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TABLE OF CONTENTS

JONES, DONALD F., Gene action in heterosis 93

BAYLOR, MARTHA BARNES, DONALD D. HURST, SALLY LYMAN ALLEN, and ELIZABETH TEEGARDEN BERTANI, The frequency and distribution of loci affecting host range in the coliphage T2H 104

TANTAWY, A. O., Genetic variance of random-inbred lines of *Drosophila melanogaster* in relation to coefficients of inbreeding 121

NANNEY, DAVID L., Inbreeding degeneration in Tetrahymena 137

YOST, HENRY T., JR., and ROBERT N. BENNEYAN, The effects of combined radiations on crossing over in *Drosophila melanogaster* 147

RUSSELL, LIANE BRAUCH, and MARY H. MAJOR, Radiation-induced presumed somatic mutations in the house mouse 161

KIRBY-SMITH, J. S., and DORIS L. CRAIG, The induction of chromosome aberrations in Tradescantia by ultraviolet radiation 176

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GENE ACTION IN HETEROSIS

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THE immediate superiority of crossbred offspring over their inbred parents or their inbred offspring, heterosis, is a manifestation of gene action. Since information concerning the nature of genes and their effects is accumulating rapidly it is understandable that knowledge of heterosis is in a formative stage. There are two general interpretations of heterosis: the accumulation of the effects of favorable dominant genes at different loci, and the interaction of different alleles at the same locus. The first hypothesis is generally understood as dominance, the second as superdominance or overdominance.

The important difference between these two hypotheses should be clearly understood. They both assume interaction between alleles. In the dominance hypothesis the interaction is between alleles at different loci. These effects can be accumulated in the heterozygous condition and fixed in the homozygous condition. In overdominance the reaction is between alleles at the same locus. These effects can be accumulated in the heterozygous condition but cannot be fixed in the homozygous condition. Therefore, the important difference in these two concepts is whether or not favorable effects can be recombined in one true breeding strain which will be relatively uninfluenced by the type of mating.

Mendelism and linkage provided a simple explanation for an accumulation of favorable effects in hybrid offspring and overcame the objections that had been raised against dominance shortly after Mendel's principles first became known. Further evidence from population genetics has raised additional objections as outlined by HULL (1952), CROW (1952), LERNER (1954), ROBINSON, COMSTOCK, HARVEY (1955), and others, to the dominance hypothesis that need careful consideration. Additional experimental evidence is needed to arrive at a decision in this matter.

The published report of the Iowa Conference on Heterosis, edited by GOWEN (1952), and of a similar discussion in England under the leadership of MATHER (1955), give a comprehensive review of investigations bearing on this subject and much new evidence. EAST (1936) gave a theoretical interpretation of overdominance resulting from the accumulation of dominant effects of alleles at the same locus. Compound genes with multiple effects and completely linked, or very closely linked, genes in the repulsion or transphase give the same result. Additional evidence has been presented by several investigators by correlating the amount of heterosis with the degree of heterozygosity and from mutant genes originating in inbred lines. New evidence is presented here from mutant genes extracted from crosses showing heterosis and from heterozygous alleles in backcrossed progenies rendered highly homozygous at all other loci. Convincing evidence of dominant genes important in heterosis has been given by EMERSON (1952), ROBBINS (1952), and WHALEY (1952).

HETEROZYGOSITY AND HETEROSIS

One of the unanswered questions is how much of the dominance effect is due to non-allelic gene interaction. There is abundant evidence showing the relation between the degree of heterozygosis and the expression of heterosis. STRINGFIELD (1950) compared four stages of heterozygosity in maize in yield of grain, time of maturity, and height of stalk, as a measure of the amount of heterosis. Inbreds from different varieties after ten or more generations of inbreeding intercrossed among themselves were used to represent 0 degree of heterozygosis; the F_2 generation from crosses of two inbreds, intercrossed among themselves, and the backcrosses of F_1 by their parental inbreds, represented 50 percent heterozygosis; the combination of two single crosses (A × B) (A × C) having one inbred in common represented 75 percent heterozygosis, and three combinations of two, three, and four inbreds represented 100 percent heterozygosis. STRINGFIELD concluded that "the expression of hybrid vigor does not follow a linear trend as equal increments of new dominants are added. The deviation from linearity was slight when yield was measured, but the other measures strongly suggest that the regression is curvilinear". SENTZ, ROBINSON and COMSTOCK (1954) compared five degrees of heterozygosis in a similar manner and obtained a proportionately higher expression of heterosis at the 25 percent heterozygosis level. This amount of hybridity was obtained by backcrossing twice in succession to each inbred parent that made up the original cross. At the other degrees of heterozygosis the relations were not essentially linear. The 75 percent point was frequently too low and never significantly too high. The authors consider this evidence to indicate an interaction of genes at different loci.

How accurately degrees of heterozygosity can be related to the amount and source of heterosis is difficult to measure. Interactions between genes at different loci occur in the homozygous as well as in the heterozygous condition. In the heterozygous condition there are simply more different genes involved. With a large number of genes, each contributing a small amount to the total effect, the accumulation would be gradual. Duplicate genes make their largest contribution at the lower degrees of heterozygosity while complementary genes add more at higher concentrations. Thus, the two types of gene action would tend to balance each other.

INTERACTION BETWEEN ALLELES

Cases of heterosis resulting from assumed single gene differences have been reported. These have been summarized by HAGBERG (1953). Spontaneous or induced mutations occurring in inbred and relatively homozygous material have been crossed back with their normal homozygous parental lines and the assumed monofactorial heterozygote has been compared with the homozygous normal and homozygous mutant. Where the homozygous mutant is lethal or severely handicapped, the comparison, of course, is made between the homozygous and heterozygous normal. In many cases, in a wide diversity of plant and animal material, the heterozygote is superior in some measurable character.

The writer (JONES 1945) studied five spontaneous mutants in maize and found the heterozygous plants to be significantly superior to the original normal line in one or more respects. The mutations were all of a degenerative nature. They were of the

type that would normally be at a distinct reproductive disadvantage under conditions of natural selection. These mutations affected leaf width, plant height, chlorophyll content, and time of flowering, and were considered to be a somewhat random sample of the degenerative changes that occur regularly in this species.

Variations of this kind occurring in a naturally cross-fertilized species like maize may be maintained in the heterozygous condition indefinitely but are rapidly eliminated by self-fertilization. Since crosses of the surviving normal lines, after these deleterious recessive genes are eliminated, are as vigorous and productive as the original population, or more so, and again show the same reduction on further inbreeding, obviously this type of variation can have little or no effect on heterosis as generally expressed in crosses of homozygous lines. They are of considerable importance in reproductive ability and adaptation in naturally cross-fertilized species. The appearance of these deleterious recessive characters in individuals when naturally cross-fertilized species are first inbred contributes directly to their reduced viability and reproductive efficiency, and usually furnish the basis for much of the apparently unfavorable effects of inbreeding.

One of the mutant characters studied, crooked stalk, was also noted to be accompanied by an obscure chlorophyll change. At early stages of growth the young leaves at the top of the plants are lighter in color. This condition, called pale top, was later found to be separable from the crooked stalk. Therefore, more than one mutation had occurred spontaneously in this inbred line. For this reason it was suspected that the heterosis apparent in the original cross of mutant and normal was not the result of a single gene difference. From this cross recessive pale crooked plants were recovered as well as homozygous normal plants. A comparison was made in height of stalk, and in weight of grain between the original mutant pale crooked and the recovered pale crooked plants as segregants from the cross with the normal line in which these mutations occurred, and with the cross of original and recovered lines. In this comparison all three lots were genetically alike for all visible characters. They were all mutant. The question to be answered is do they differ in growth and productiveness. If so, could this be due to genes obtained from the normal line that were not present in the original mutant line? The results are given in table 1. The comparison was made in a 3 × 3 Latin square replicated three times. The letters identify the different entries.

In all comparisons shown in tables 1 to 3 seed from several hand pollinated plants in each lot was bulked and planted in rows ten feet long and three feet between rows. An excess of seed was planted and thinned at an early seedling stage to ten plants per plot. The plants were measured for height to the tip of the unbroken tassel as soon as all pollen was shed. Ears were harvested at maturity and dried to uniform moisture content. Growing conditions were favorable and a good stand of plants was obtained in nearly all plots. In some cases the complete number of plants was not measured because of broken tassels. In these cases there was no unevenness in stand. All of the results shown in the tables are analyzed for significance by the standard methods. The results are given with each table.

The recovered homozygous pale crooked and the first generation cross of the original and recovered mutant lines are both significantly taller than the original

TABLE 1

A comparison in plant height of original and recovered mutant lines of maize, pale and crooked, in self-fertilized and intercrossed progenies

Field arrangement and average height

P 51.0	Q 58.9	R 58.8	Q 57.5	R 58.0	P 54.3	R 59.0	P 54.8	Q 58.6
Q 54.9	R 58.1	P 54.9	P 53.9	Q 55.7	R 59.9	P 55.8	Q 57.2	R 57.3
R 52.3	P 53.1	Q 57.6	R 56.2	P 50.5	Q 58.4	Q 54.4	R 56.7	P 56.0

Code	Progenies	Average height of plant in inches
P	Original Pale Crooked Selfed	53.8
Q	Recovered Pale Crooked from Cross	57.0
R	Original × Recovered F_1 Cross	57.4

Analysis of Variance

Item	DF	SS	MS	F	P
Rows	2	15.30	7.65	4.93	$<.05$
Columns	8	54.63	6.83	4.40	$<.01$
Varieties	2	69.22	34.61	22.31	$<.001$
Inbred vs. crosses	(1)	(68.69)	(68.69)	(44.32)	$<.001$
Within crosses	(1)	(0.53)	(0.53)	(0.34)	$>.5$
Error	14	21.80	1.55	1	

Q and R taller than P at probability .95 (from t_R test).

Q and R (crosses as a group) taller than P (inbred) at probability .999 (from analysis of variance).

mutant line. The same comparison was made between the original normal and the recovered normal and between crosses of original normal by the original mutant line and the recovered normal and recovered mutant line, and also with the cross of original normal and recovered normal. In this last cross the mutant genes are not involved. The results are given in table 2. The comparison is made in a 6 × 6 Latin square arrangement of plots. Just as the recovered mutant line came out of the cross taller than it went in, so also did the recovered homozygous normal line. The cross of original normal and pale crooked gave the same increase in height (six percent) as when previously crossed several generations earlier, as reported in 1945. The cross of recovered normal by recovered pale crooked also showed the same increase in height. Likewise, the cross of original normal by recovered normal, where the mutant genes were not involved, gave the same increase in height.

Two normal lines (O and J in table 2) from the same source have been continued by self-fertilization. These do not differ in any measurable degree. Crosses between normal lines were compared in the original report. These showed no increases in crosses. A further test is needed.

Yield of grain of all of these progenies was also compared. The differences are in the same direction but no significant differences were obtained and the results are not reported here. Yield of grain is very difficult to measure in inbred strains of maize. Small environmental effects may determine whether or not a plant produces seeds obscuring small genetic differences.

TABLE 2

A comparison in plant height of original normal inbred lines of maize and normal lines recovered from a cross with mutant lines, pale and crooked, out of the same inbred line

Field arrangement and average height

J 73.3	K 75.6	L 78.6	M 79.0	N 79.7	O 72.3
M 78.6	J 71.3	O 75.4	K 79.6	L 79.6	N 78.6
K 76.2	L 77.1	N 78.2	J 72.0	O 76.3	M 77.5
O 71.4	M 78.1	K 78.9	N 78.6	J 74.3	L 79.0
N 76.5	O 72.9	J 75.9	L 79.0	M 78.3	K 76.5
L 75.0	N 74.8	M 78.8	O 72.2	K 75.7	J 69.5

Code	Progenies	Average height of plant in inches
O	Original Normal	73.4
J	Original Normal	72.7
K	Recovered Normal from Cross	77.1
L	Original Normal × Original Pale Crooked, F_1 Cross	78.1
M	Recovered Normal × Recovered Pale Crooked, F_1 Cross	78.4
N	Original Normal × Recovered Normal, F_1 Cross	77.7

Analysis of variance

Item	DF	SS	MS	F	P
Rows	5	29.16	5.83	5.00	$<.005$
Columns	5	39.42	7.88	6.76	$<.001$
Varieties	5	187.17	37.43	32.08	$<.001$
Inbreds vs. crosses	(1)	(180.19)	(180.19)	(154.01)	$<.001$
Within inbreds and crosses	(4)	(6.99)	(1.75)	(1.50)	$>.2$
Error	20	23.34	1.17	1	

K, L, M, and N taller than O and J at probability .95 (from t_R test).

K to N (crosses as a group) taller than O and J (inbreds as a group) at probability .999 (from analysis of variance).

It is obvious that genetic differences other than the visible mutations are involved in these comparisons of self-fertilized and intercrossed lines. Both the mutant and the normal lines came out of the first cross taller than they went in. These effects are dominant in crosses since the taller growth is shown by all of the intercrosses.

EVIDENCE FROM BACKCROSSED LINES

Additional evidence has been sought by a somewhat different experiment in which an attempt was made to compare homozygous and heterozygous single gene differences in backcrossed lines. For this experiment two inbred strains were selected that differed in easily visible gene markers. The genes involved are yellow and white endosperm color, red and white cob color (alleles of the *P* pericarp color gene), and normal and glossy seedlings. One of the two inbreds showing different alleles of these three genes is Connecticut 243, one of the original Leaming inbreds (designated 1–6 in earlier publication). This line had been continuously self-fertilized for over 35 genera-

tions when the experiment was started. The other line is Connecticut 20 out of Burr White, and continuously self-fertilized more than 20 generations. C243 is *VY*, *PP*, *gl gl* in composition, and C20 is *yy*, *pp*, *Gl Gl*. The first generation hybrid plants of this combination show the usual increase in height and productiveness characteristic of crosses of inbred lines out of varieties of different type. The first generation hybrid was backcrossed by both parental lines and plants showing each dominant gene marker were continuously backcrossed in successive generations by the line that was recessive for the marker gene in each case. Three lines were established, each approaching the recurrent recessive parent in all visible characters except for the one dominant gene marker that was selected in each generation and maintained in the heterozygous condition. The yellow endosperm and red cob lines were each backcrossed continuously by the C20 line, recessive for these alleles. The non-glossy line was also backcrossed continuously by the C243 line, recessive for the glossy leaf character determiner.

The plan of the experiment was to backcross long enough to restore the visible characteristics and level of vigor of the recurrent parent, and then make a careful comparison in measurable characters between individuals or progenies heterozygous dominant and homozygous recessive from the same parents.

These three gene markers were selected because they have no differential effect on growth or productiveness as far as known in the dominant or recessive condition. They are, therefore, suitable for comparing the effect of the homozygous and heterozygous states. Yellow seeded and white seeded varieties of maize are about equal in numbers throughout the world. This gene controls the amount of vitamin A precursor stored in the seed, but has no effect on the growth of the plant as far as it can be measured. Likewise, no differences in yield or other measurable characters have been established for red cob or white cob varieties, or between normal and glossy seedlings. The glossy character affects the water shedding ability of the leaf surface and might be considered to be an unfavorable character. Normal plants are pubescent and bluish green in color of the seedling leaves and shed water readily. Glossy plants lack this pubescence in the early seedling stage. However, many species of grasses are normally glossy and a number of standard inbreds widely used in the production of hybrid corn are glossy. Glossy plants become normally pubescent over most of the leaf surface after the early seedling stage but can be easily distinguished in small areas until flowering.

These three genes in their alternate alleles can be assumed to have little or no effect on growth and productiveness. The evidence presented here bears this out. They also represent a type of gene differences that characterizes varieties. It is just such varietal differences that were assumed to contribute to heterosis by EAST and by SHULL in their original hypothesis of a stimulus from heterozygosity itself.

While the three genes studied comprise a very small sample of such gene varietal differences, they are thought to represent a characteristic random sample of such differences. All three genes show clear-cut Mendelian segregation and recombination and have been located on chromosome maps.

An additional source of the *Y* endosperm color gene was obtained from a third unrelated inbred designated as Indiana P8. This line was selected as a source of the dominant *Y* gene as it had a darker yellow color. The cross of P8 × C20 was also

backcrossed by C20 and carried along in the same way as the cross of C243 and C20. In both backcrosses the yellow color was diluted rapidly and after a few generations the difference in color between *Yy* and *yy* seeds on the same ear was quite faint but could be easily distinguished on a blue background in strong daylight. The color separation was always checked by different persons and, since only yellow seeds were used for further backcrossing, faulty classifications or errors due to heterofertilization were eliminated in each generation if they occurred, and would have no bearing on the results obtained. The darker color of the P8 source was evidently due to modifying genes which were quickly lost as both backcrossed series appeared to be the same in depth of color.

The backcrossed lines approached their recurrent parents closely in size, time of maturity, and other visible characters in the sixth to eighth generation. About 15 plants were grown in each backcrossed progeny in each successive generation. The homozygous inbreds were used as the pollen parent. Plants used for cross pollination were selected at random in the field as far as this can be done. Weak plants produce no ear shoots and naturally cannot be used for propagation as in any inbreeding or backcrossing experiment. The inbred lines used for backcrossing were always propagated by self pollinated plants.

Selection of heterozygous plants or seeds in each generation enforced heterozygosity upon the one locus involved in each case in each generation, and presumably this locus also carried with it a section of chromosome on each side of the marker gene maintained also in the heterozygous condition. Without this enforced heterozygosity random mating would reduce the expected heterozygosity to less than two percent in six generations of backcrossing on the average. Since all of the backcrosses were visibly larger and different from their recurrent inbred parents until after six generations this enforced heterozygosity had some effect, and genes affecting heterosis are presumably present in these regions of the chromosomes.

Backcrossing was continued for 11 or more generations, until no visible differences were apparent between the original self-fertilized recurrent parental line and the two backcrossed progenies in each case, one heterozygous dominant and the other homozygous recessive for the gene marker used in each case. Homozygous progenies were also included in the comparison grown from homozygous recessive plants self-fertilized from a previous backcrossed generation.

For the comparison of the glossy and normal plants backcrossed by C243 three progenies were grown in a 3×3 Latin square replicated five times. The three lots of seed planted came from several ears in each case and were as follows: the original glossy inbred, C243, continuously self-fertilized; glossy plants from the 12th generation of backcrossing, backcrossed once more by glossy C243; normal plants in the 12th generation backcrossed once more by the glossy inbred. This last progeny gave an equal number of normal and glossy plants which could only be separated after planting. Dominant and recessive plants were marked in the field and measured for height. No significant differences were found. The parental, continuously selfed glossy inbred measured 78.2 inches in average height at maturity. The homozygous backcrossed glossy progeny was slightly taller measuring 80.6 inches in height. The segregating backcrossed progeny averaged 81.4 for both dominant and recessive plants. When separated into two groups the homozygous recessive glossy plants averaged

82.1 and the heterozygous dominant normal plants averaged 80.3 inches in height. These differences are not significant and show no superiority of the plants heterozygous *Gl gl* compared with the plants homozygous recessive at this locus. In this last comparison the homozygous and heterozygous seeds were produced on the same plants and in equal numbers, thus avoiding any differences in germination or vitality of seed from any source.

Yield of grain was also compared in this series. Many of these long inbred plants produced no ears and no significant differences were recorded. All of the backcrossed lines yielded more than the continuously self-fertilized line. Although slightly shorter in height the heterozygous normal plants yielded considerable more than the homozygous glossy plants from the seed grown on the same plants. However, the average yield for both kinds of plants in this segregating progeny was only 73.2 grams per plant compared to 71.2 grams for the homozygous glossy plants equally backcrossed. Plot yields varied from 31.7 to 114.0 in grams per plant, and these results cannot be relied upon. Some other measure of productiveness is needed in a comparison of inbred plants such as these.

A similar comparison is made between plants heterozygous and homozygous for the gene markers *Yy* and *Pp*. Nine different progenies were grown in a 9 × 9 Latin square. The results are given in table 3. In this series the other parent, C20, of the original cross was used as the recessive recurrent parent. Eight different lots of backcrossed plants are compared with the original inbred parental line. This parental line was propagated by a single selfed ear in each generation from the same progeny as used for backcrossing.

After 13 generations of backcrossing white cob plants in this segregating generation were selfed and grown as lot B in table 3; other white cob plants were again backcrossed and grown as lot D. The seed from red cob plants also backcrossed were grown as lot C. In each case seed from several plants of the same genotype was mixed. The backcrossed red cob plants produced red and white cob plants in equal numbers. These could not be separated in the field at the time the plants were measured for height. Many plants produced no ears. These three lots averaged B 73.3, C 74.2 and D 75.0 inches in height compared with A 72.3 for their continuously self-fertilized parental line. The difference between the backcrosses as a group and the inbred parent is highly significant. The differences between the various backcrossed progenies are not significant. As in the other series, the backcrossed progenies are slightly taller than the inbred but there is no difference between the plants heterozygous dominant and homozygous recessive for the *Pp* marker gene.

The most critical comparison is between the plants grown from yellow and white seeds produced on the same ears. These seeds were separated before planting and the comparison can be made between progenies in which the plants are either all heterozygous *Yy*, E and G, or all homozygous *yy*, F and H. As stated previously, the dominant *Y* gene was derived originally from two different sources, inbred lines C243 and P8. In the series from C243 the two lots, E and F, averaged exactly the same in height. In the two lots from P8, G and H, the plants from yellow seeds are slightly taller but not significantly so. Plants from white seeds again backcrossed by the white seeded parent, I, also show no significant differences from the yellow seeded plants. In every case the backcrossed lines are taller than the original inbred line. As a group the differences are

TABLE 3

A comparison in plant height of an original self-fertilized line with recovered backcrossed lines, heterozygous and homozygous for the marker genes Pp *and* Yy, *derived from unrelated lines*

Field arrangement and average height

A 69.0	B 72.3	C 71.8	D 74.5	E 74.2	F 74.8	G 75.0	H 72.1	I 70.0
B 71.8	C 74.6	E 73.7	G 71.2	D 76.4	I 72.7	F 75.4	A 73.3	H 70.5
C 73.0	D 75.6	F 73.8	A 72.4	H 70.9	G 72.7	I 75.8	E 72.3	B 71.8
D 70.6	H 72.4	A 70.7	B 71.5	F 73.5	E 74.7	C 73.3	I 74.5	G 73.1
E 70.3	G 77.4	B 74.4	I 75.6	C 76.3	H 76.1	D 76.1	F 75.2	A 69.3
F 69.3	I 76.8	H 75.9	E 75.3	B 71.9	D 75.9	A 76.9	G 76.8	C 69.1
G 73.0	F 76.4	I 74.7	C 77.8	A 71.5	B 74.1	H 76.6	D 76.4	E 73.4
H 69.8	E 77.3	G 78.2	F 76.1	I 73.9	A 72.7	B 75.0	C 76.0	D 74.2
I 71.0	A 75.3	D 75.6	H 76.3	G 73.7	C 75.6	E 77.8	B 77.2	F 74.0

Code	Progenies	Average height of plant in inches
A	Original inbred line C20, *yy pp*	72.3
B	Recovered selfed line from backcross, white cob, *pp*	73.3
C	Segregating backcrossed line, red and white cob, *Pp* and *pp*	74.2
D	Recovered backcrossed line, white cob, *pp* × *pp*	75.0
E	Heterozygous backcrossed plants from yellow seeds, C243, *Yy*	74.3
F	Homozygous backcrossed plants from white seeds, C243, *yy*	74.3
G	Heterozygous backcrossed plants from yellow seeds, P8, *Yy*	74.6
H	Homozygous backcrossed plants from white seeds, P8, *yy*	73.4
I	Recovered backcrossed line, white seeds *yy* × *yy*	73.9

Analysis of variance

Item	DF	SS	MS	F	P
Rows	8	70.53	8.82	3.65	$<.005$
Columns	8	192.26	24.03	9.94	$<.005$
Varieties	8	46.03	5.75	2.38	$<.05$
Inbred vs. crosses	(1)	(25.36)	(25.36)	(10.48)	$<.005$
Within crosses	(7)	(20.67)	(2.95)	(1.22)	$>.2$
Error	56	135.42	2.42	1	

D taller than A at probability .95 (from t_R test).

B to I (crosses as a group) taller than A (inbred) at probability .995 (from analysis of variance).

highly significant. This difference cannot be attributed to the heterozygosity of the visible marker genes involved.

DISCUSSION

The assumption that naturally occurring visible mutations in highly homozygous inbred lines of maize involve only single gene changes is not valid as indicated by the writer (1952). The evidence for this conclusion is presented here. Additional critical evidence has been given by SCHULER (1954) JINKS (1955), and SCHULER and SPRAGUE (1956). Other gene changes occur at or near the time the visible mutations appear or all heterozygosity of genes affecting growth cannot be eliminated by continuous self-fertilization.

Although there is evidence for an overdominance effect from a study of variability in cross-fertilized organisms as presented by HULL (1952), CROW (1952), LERNER (1954), and others, specific cases of single gene superiority or overdominance are remarkably few and even these cases may be due to multiple gene effects as shown by the cases given here that have been more critically analyzed.

The mutant tobacco described by the writer (1921) is a good illustration of single gene superiority. The change involved the reaction to length of day. The mutant plants were unable to flower in the normal summer day but flowered and produced seed in a shortened day. The visible change was due to a spontaneous mutation from a single dominant to a recessive condition. The recessive mutant was wholly unable to survive under natural conditions in the long day at northern latitudes. The heterozygote of normal and mutant alleles was better able to survive than the homozygous normal since the plants produced more leaves, flowers, and seeds under the same conditions. This is a clear case of single gene superiority but the heterozygote shows no acceleration of growth rate. It merely extends the growing period longer to produce a larger amount of material and greater reproductive ability. Whether other genes are involved in the larger growth and greater reproductive ability of the heterozygous plants is not known. This has not been examined by the recovery tests as done here, but whether or not other genes are involved has little bearing on the question at issue, since number of leaves in tobacco is not influenced by heterosis to the extent shown in this case. Whether this is a case of heterosis is a matter of definition.

A similar situation has been reported by QUINBY and KARPER (1946) in sorghum. A single gene for time of maturity in the heterozygous condition when acting with other genes, also regulating maturity, greatly increases the total amount of growth. As in tobacco there was no increase in the rate of growth. Theoretically, many genes could act in this way. It is surprising that so few instances have been found (see LERNER 1954; PONTECORVO 1955).

Many genes show dosage effects where the productiveness of the gene is correlated with the numbers of dominant alleles present. In any case where the diploid or higher number of normal alleles at the same locus produces an excess of essential ingredients, thereby reducing the effectiveness of other genes involved in a reaction system, the hemizygote or heterozygote would be superior. There is every reason to expect that conditions of this kind would be selected for in cross-fertilized organisms. Apparently the genes of most importance in physiological processes cannot be easily identified in the higher plants and animals where heterosis has been mainly studied.

SUMMARY

A sample of two different types of genes in maize (1) unfavorable degenerative mutants, and (2) varietal differences with no effect upon growth and productiveness have been compared in the homozygous and heterozygous condition. These two types of genes provide most of the visible variation found in this naturally cross-fertilized plant. Neither type gives any evidence for overdominance.

Both types of genes show dominance. The heterozygous normal is equal to the homozygous normal in all visible characters in the degenerative mutant series (1)

and the presence of color is dominant over no color, and pubescence over non-pubescence in the varietal difference series (2).

The evidence shows that other genes or some unknown factors are involved in producing heterosis, since all backcrossed and intercrossed progenies are taller than the corresponding continuous self-fertilized progenies.

If genes are involved in this heterosis they have no visible effects by which they can be identified by the techniques that have been used so far.

It is possible that these unknown genes may show overdominance but this has not been proved.

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THE FREQUENCY AND DISTRIBUTION OF LOCI AFFECTING HOST RANGE IN THE COLIPHAGE T2H[1]

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THE host-range phenotype has provided a very useful and productive tool in the studies of mutation and recombination in the bacteriophages. It, therefore, becomes of interest to search for additional host range mutants, to study the distribution of these markers in the T2 linkage groups, and, finally, to explore the mechanism of phenotypic expression of these genes at a level where a direct chemical approach can be made.

LURIA (1945) described host range as a genetic character of T2 which is dependent upon the adsorption of the phage to the bacterial cell. Using the adsorption and efficiency of plating of phage as phenotypes, HERSHEY and DAVIDSON (1951) found several alleles at the known *h* (host-range) locus as well as a second locus affecting the host range.

The presence of the *h* allele of the *h* locus affects the initial adsorption of the phage to the bacterial surface in the case of T2 (LURIA) and B/2 (PUCK 1953). However, an apparent host-range phenotype may be brought about by a number of mechanisms, and it is not obvious that in a given phage-host system the mechanism will be the same for all mutants. An example of an alternate mechanism is certain *r* plaque type mutants in the *r*II region of T4 which, unlike the wild type, are not able to form plaques on the cell K12 (λ), (BENZER 1955). These mutants adsorb to K12 carrying λ, kill the cells but promote very little, if any, lysis.

In studies of genetic recombination, the *h* locus was used originally by HERSHEY and ROTMAN (1949) and that work has become a classical marker in the study of T2 genetics. Host range has been found to be a useful marker in other lytic coliphages (BRESCH 1953; FRASER and DULBECCO 1953; ADAMS 1952 for T1, T3, and T5, respectively). The results of FRASER and DULBECCO (1953) closely parallel those described here and will be discussed later.

MATERIAL AND METHODS

Origin of bacterial and viral strains

All bacterial and viral strains were derived from the strain *Escherichia coli* B (S and H) and B/2 (2bc), and the phages T2 and T2*h* which were obtained through the generosity of Dr. A. D. Hershey. The Hershey and Luria strains of B, B/2, T2,

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TABLE 1

Origin of the mutants and their host range

	B	B/2	B/2/*h*	B/2/*h*,*h*′
T2	*r*	0	0	0
1*ht*	*r*	vtu, sm	0	0
2*ht*	*r*	tu, *r*	0	0
3*ht*	*r*	cl, *r*	0	?
T2*h*	*r*	cl, *r*	0	0
*h*1*ht*	*r*	cl, *r*	vtu	?
*h*2*ht*	*r*	cl, *r*	tu	?
*h*3*ht*	*r*	cl, *r*	cl	vtu
T2*h*′	*st*	cl, *r*	cl	0
h′1*ht*	*st*	cl, *r*	cl	vtu
h′2*ht*	*st*	cl, *r*	cl	tu
h′3*ht*	*st*	cl, *r*	cl	cl, *r*

All plaques on B/2/*h* small.

0—no transfer, no plaques; vtu—very turbid transfer and plaques; tu—turbid; cl—clear; *st*—starred plaque form; *r*—r and r^+ can be distinguished; sm—small; ?—maybe some plating ability.

and T2*h* differ in respect to resistance and host range. T2L contains several mutant *ht* loci as shown by crosses with T2H. All strains used in this study were derived from Hershey strains. The two indicator cells, B/2/*h* and B/2/*h*,*h*′, were isolated after selection with T2*h* for resistant mutants in populations of B/2. The origin of the phage mutants and their host ranges is shown in table 1. All stocks used in recombination studies were grown in the cell S.

The genetic cross

A genetic cross is effected by multiply infecting bacteria with two parent phages, allowing growth until a single cycle of growth occurs, then scoring the progeny of the burst either by direct plating on indicator cells or by replication to the indicator cells from platings on sensitive cells. Total multiplicities (number of phage per bacterium) of seven to ten and ratios of the two parents of 0.6 to 1.0 were used; data from crosses having lower multiplicities or lower parental ratios were not used. Higher multiplicities caused lysis without phage production. Adsorption was usually done in buffer on the cell H, followed by the passage through T2 antiserum to remove free phage. Buffer: $Na_2HPO_4 \cdot 7H_2O$, 5.7 gm; KH_2PO_4, 1.5 gm; NaCl, 4 gm; K_2SO_4, 5 gm; 1 liter deionized H_2O. Autoclave together. Add 10 ml, 0.1M $MgSO_4$; 10 ml, 0.01M $CaCl_2$; 1 ml, 1 percent gelatin.

Methods of scoring progeny

In the course of the study there has been a progression of scoring techniques. Results using two of these, direct plating and replication, are included in this report. Where the recombinants were identified by direct plating on indicator cells, the progeny of a cross were first preadsorbed on the sensitive cell H at a phage/cell ratio of 0.1, then diluted by a factor of 10^4 or more before plating on the indicator cell. Preadsorption raises the e.o.p. to unity and eliminates phenotypic mixing (HERSHEY,

et al. 1951; NOVICK and SZILARD 1951). However, direct plating and plating on mixtures of sensitive and indicator cells have two major disadvantages: (1) the indicator cell itself may influence the final phenotype; and (2) by the time sufficient growth has occurred to form visible plaques, selection of plaque mutants may, in some cases, have completely obscured the properties of the initiating phage. These plaques cannot be used as a source for a stock of the recombinant.

To eliminate such difficulties and to increase the number of progeny tested for the entire host range, the replica technique of LEDERBERG and LEDERBERG (1952) for detecting bacterial mutants was modified for use with plaques. Plaques formed on sensitive cells are transferred by means of velvet to a plate of thin but confluent indicator cells. The cell layer is obtained by a heavy inoculum (5×10^8 per cc) into 1.5 percent agar poured onto an ordinary agar plate and incubated at 37°C previous to replication. Four hours preincubation has been found to be optimum for B/2. Both the replica plate and the master plate are chilled to prevent peeling of the top layer agar during the replication process. A positive transfer is a lytic area on the specialized cell resulting from virus transferred by the replication process from a plaque on the generalized cell. The transfer will be further described in terms of its turbidity as very turbid, turbid, slightly turbid and clear.

Efficiency of plating as used in this study refers to the ratio of the number of plaques formed on the indicator cell to the number formed on the sensitive cell, S.

Once the replica technique was developed, the progeny of all subsequent crosses were scored by this technique. This constitutes the major portion of the crosses to be reported. All recombinants, from which stocks were made for use in later crosses, were taken from the master plate. The assays of parent phages were replicated concurrently with the replication of the progeny in all crosses.

Determination of phage mutants by replication

Because it became obvious that the parent stocks contained large numbers of mutants, it was necessary to determine the level of mutants in the parent stocks and later in single plaques. The stocks or suspensions of single plaques were plated on the sensitive cell such that 500 to 1000 plaques appeared per plate. After incubation to a point where the plaques were just visible, replication was effected to the indicator cell. The plaques were replicated early in their growth to avoid false positives caused by plaque mutants which are confusing only on crowded plates. Whenever possible, plaques from the master plate were picked to serve as the source of mutant stocks. The major portion of new markers used in this study were derived from mutants isolated in this nonselective manner. The mutants have never been exposed, except indirectly, to indicator cells. The number of mutants in the stocks (0.05 percent to 0.1 percent) determines the level of definition between loci.

The percent recombination was obtained by determining the fraction of recombinants in the total population of progeny. The observed values were corrected by the formula of LENNOX, LEVINTHAL, and SMITH (1953) for multiplicity of infection and unequal parental ratios (see also DOERMANN and HILL, 1952). Multiplicity of infection was determined by cell killing, and the output ratio of parental types was determined by differentially marking the two parents for plaque type. Where the two parents were not so marked and were indistinguishable, the ratio had to be

determined by separate assays of the input components. The chief experimental factor contributing to the high variance between crosses is lysis-from-without, which selects for cells receiving the lowest multiplicities.

Nomenclature of mutants

HERSHEY and DAVIDSON (1951) studying the genetics of host range of T2 found mutants of two different phenotypes; one, designated as h^c, gave clear plaques and a high e.o.p. on the indicator cell B/2, and the other, designated as h^t, gave turbid plaques and a low e.o.p. on B/2. The mutants to be described in this work are similar to the second of the above types; they give very turbid plaques and a very low (0.01) e.o.p. on B/2. Consequently, the above designation will be retained. The mutated loci will be designated as *ht* markers and the different loci distinguished by numerical subscripts. The superscript *t* of the HERSHEY and DAVIDSON nomenclature has been lowered as a convenience in note taking where *t* is easily confused with +. The h^c allele, and the locus which contains it will be called the *h* locus in accordance with a large literature now extant in T2 genetics. As the *ht* mutants were selected, they were numbered consecutively, and thus overlap the numbering of the HERSHEY and DAVIDSON h^t mutants, 1 through 5. These mutants have never been tested against ours and it is not known whether any of the two series involve the same loci. Since it is now necessary to refer to the origin of any markers, the *ht* markers reported here can be called the M or Michigan series.

The other phenotypes referred to in the study are *r* (rapid lysis) and *m* (minute). These are previously located markers (HERSHEY and ROTMAN, 1949) and were used to map the *ht* loci. The only unique plaque morphology phenotype found in this study was "star" which was observed concurrently with the host-range variant T2*h'*.

RESULTS

Additive effect of ht *loci*

A phage containing a single *ht* mutation can lyse B/2 which the wild-type is unable to lyse and gives a very turbid lytic area upon replication and a very low

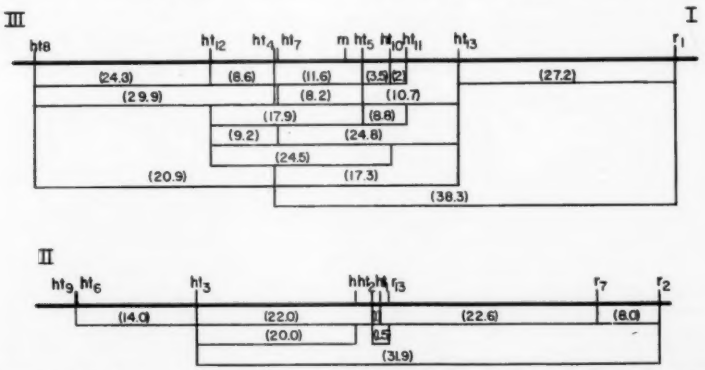
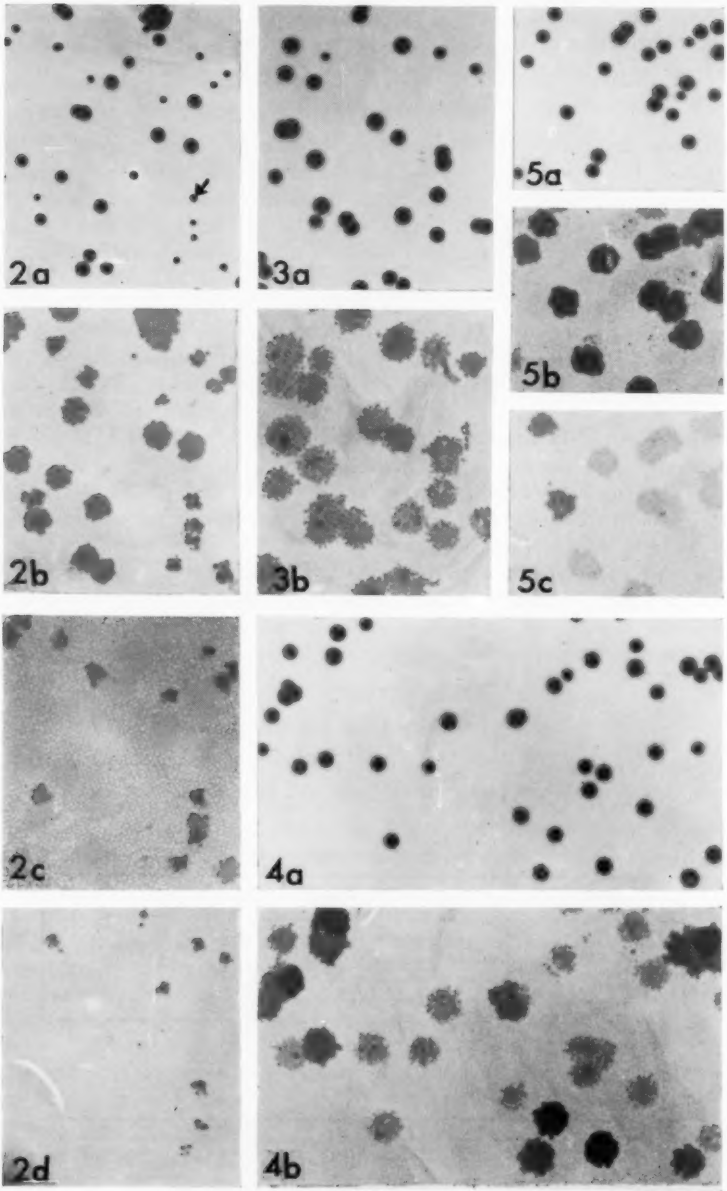


FIGURE 1.—Linkage map of T2H, showing the Hershey markers r_1, r_2, r_7, r_{13}, and *m* in relation to newly isolated *ht* markers.



FIGS. 2a–5c

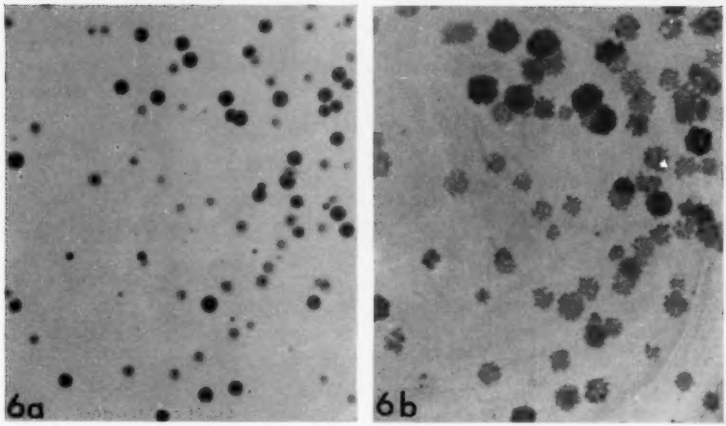


FIGURE 6a.—Plating of progeny of cross of *rht* with r^+ht on S.

6b.—6a replicated to B/2. Note that the wild recombinant is missing and that the turbid replication of the double *ht* recombinant is clearly distinguishable from the very turbid replication of the parent phages.

e.o.p. on B/2. A mutation at any one of the *ht* loci results in the same phenotype. A phage containing mutant alleles at two *ht* loci forms a distinctly less turbid transfer on replication with a higher e.o.p. and clearer plaques by direct plating. The recombinant containing the mutant alleles at three *ht* loci, at least for some combinations of loci, replicates clearly on B/2 and is similar in phenotype to T2*h*. The one containing four *ht* alleles is clear on B/2 and may under the best plating conditions give some evidence of transfer on the cells B/2/*h* and B/2/*h*,*h*′. Thus the *ht* mutations are additive in effect (fig. 3 and 4, and table 1). The replicated progeny from a cross involving two *ht* parentals is shown in figure 6.

Forty different double *ht* recombinants have been isolated. When plated together and replicated, they are found to differ somewhat in degree of turbidity. All double

FIGURE 2a.—A mixture of phages containing the alleles h^+, *h*, and *h*′ at the *h* locus and the variant M6 plated on the sensitive cell S. Note "starred" plaque form of T2*h*′ and M6 as shown by arrow. The photograph is too small to show irregularity of edges giving starred appearance.

2b.—The above replicated to B/2. Note the h^+ phage is missing.

2c.—The above replicated to B/2/*h*. Note the phages containing h^+ and *h* are missing.

2d.—The above replicated to B/2/*h*,*h*′. Note only M6 has replicated.

FIGURE 3a.—A mixture of six stocks of different *ht* mutants plated on S.

3b.—3a replicated to B/2. All give similar, very turbid, replication.

FIGURE 4a.—A mixture of phages containing various (1, 2, and 3) doses of mutant *ht* loci plated on S.

4b.—4a replicated to B/2 showing distinctness of turbidities of different *ht* dosages.

FIGURE 5a.—A mixture of phages of the following genotypes, wild, 1*ht*, *h*, *h*2*ht*, and *h*3*ht* plated on S.

5b.—5a replicated to B/2. Note phage carrying h^+ is missing.

5c.—5a replicated to B/2/*h*. Note phage containing h^+ and *h* are missing, and note the dosage effect of *ht* in combination with *h*.

recombinants involving ht_5 produced exceptionally weak transfers, the weakness seemingly a peculiarity of the locus ht_5 which does not differ from the others when in a single dose. The double recombinant ht_3ht_4 produces exceptionally strong transfers approaching the phenotype of the triple *ht* particle. Combinations of either ht_3 or ht_4 with all other *ht* loci do not give similar strong phenotypes which, therefore, seems to be a property of the particular combination.

Only two triple *ht* phages, $ht_2ht_3ht_4$ and $ht_2ht_{10}ht_{11}$, have been prepared as stocks and studied in detail. Both give completely clear replications on B/2; some triple combinations do not show this strong additive effect. A study of the dosage effect awaits more genetic study and physiological analysis.

The phenotype is, therefore, determined by the number of certain mutant *ht* loci present within a single particle. It is also dependent on the particular allele present at the *h* locus which determines the base line at which the host range starts. The *ht* mutations add to this base line by enabling the phage to form plaques on an entirely new cell. The relations of the *ht* dosage and the *h* alleles are shown in table 1.

The series progresses through three step gradations on the three indicator cells, as arranged in table 1 (see also fig. 2, 3, 4, and 5). The series terminates in the variant M6 which forms clear plaques on all cells. The order has never been interrupted by genetic recombination within the M6 and *ht* series. Exceptions to the pattern occur only by mutation.

Mutational variants

The viruses which were derived from direct plating on the indicator cells, B/2/*h* and B/2/*h*,*h*′ are T2*h*′ and M6 respectively, both of which form starred plaques on the sensitive cell S. In crosses between T2*h*′ and wild, two percent of the progeny produce very turbid replications on B/2 and a non-starred plaque. The turbid recombinant has been designated as ht_1. Since *h*′ arose by mutation from *h*, it might be expected the *h* would segregate out in the cross if the difference were only at an *ht* locus. Out of 3000 plaques examined, *h* was not observed. Until the reciprocal recombinant of ht_1 is identified, the product of the unknown with ht_1 will be called *h*′.

On the hypothesis that the *h* allele at the *h* locus might consist of a cluster of tightly linked *ht* loci, crosses were performed with wild and *h* using the replica technique to determine single *ht* segregants should they occur. Up to the limit of resolution, no recombination between the wild type and *h* has been observed.

A second phage variant phenotypically identical to *h*′ arose from a stock of T2$r_{13}h$. The variant showed no recombination with the known *h*′ allele, and in crosses with h^+, two percent of the progeny gave the phenotype of a typical *ht*. This was interpreted as a second isolation of the *h*′ modification.

Two separate further *h*-like variants have been isolated from T2 by screening on B/2. They show no recombination with the original T2*h* strain or T2-wild and are considered to be new isolations of the *h* allele of the *h* locus. Mutations of the *h* locus have therefore been isolated four times during the course of this work.

Properties and genetic composition of M6

The variant M6, which was isolated from a population of T2*h*′r_1 plated on the resistant cell B/2/*h*,*h*′, shows three distinct phenotypic characteristics, namely, an

increased host-range, a starred plaque which it shares with *h′*, and an instability under conditions optimum for T2. M6 forms starred plaques on the sensitive cells S and H. It forms clear plaques typically *r* in morphology on the other cells. Platings of starred plaques were found to contain a mixture of phages. Some produced starred plaques and had the same host-range as M6, while others formed typical *r* plaques and possessed a lower host-range. M6 showed no host-range or plaque mutants in about 5000 plaques examined when grown through five successive one-step growth cycles in broth, the growth tube being diluted into fresh bacteria (H) before each burst. This demonstrated that, under these conditions, M6 is not inherently unstable in a cell in which it forms starred plaques on agar.

Newly released phages from sensitive bacteria, either singly or multiply infected with M6, are extremely labile unless adsorbed onto fresh cells immediately after burst, while particles from a mixed burst of either wild or *h* are stable. Stocks of M6 prepared from confluently lysed plates, suspended in buffer, and containing 1 to 3×10^{11} particles per ml, upon storage in the refrigerator, have remained at a constant titer for more than three years. Perhaps materials from the confluent plate taken up with the phage protect it against the action of the buffer.

Particles of M6 are rapidly inactivated when diluted into buffer. The titer falls exponentially by a factor of 100 within ten minutes when aerated at 37°C. It is stable in buffer at pH5, in broth at pH7, and in distilled water. The inactivation at a high pH cannot be reversed by lowering the pH. The loss of ability to form plaques is accompanied by a loss of killing ability, but not by a loss of particle number measured by light scattering or by a loss of DNA from the particles measured by UV adsorption. Recent studies indicated that the instability may be associated with one of the modified loci in M6, namely ht_3.

From crosses involving M6 or recombinants of M6 with *h*-wild, the following mutant loci have been isolated: *h′*, ht_2, ht_3, and ht_4. These latter three mutations must have occured at the time of the formation of M6. It is not certain that the genotype of M6 has been completely described.

Frequency of the ht *mutation and derivation of new mutants*

In the course of this work, it became apparent that the different stocks of T2 contained a high level of *ht* mutants. Therefore, the number of mutant particles per parent particle was measured in stocks of different origins, as well as in populations of particles derived from single plaques. The results are seen in table 2. Out of 125,000 plaques examined, 53 mutants were found, or an average of one mutant per 2400 parent particles. The number of the *ht* mutants appeared to be similar, regardless of the genotype of the parent phage. The number of *ht* mutants in the parent stocks is about equal to the number of *r* mutants in r^+ stocks. The mutation rate toward the *ht* condition is probably of the same order of magnitude as the mutation rate from r^+ to *r* (LURIA 1953).

As in the case of the *r* mutants, it was considered likely that the high level of mutants might be a result of the accumulated mutations at many loci, all of which produce the same phenotype. Therefore, some of the mutant plaques were made into stocks. Mutants from different stocks or plaques were chosen. Eight such mutants obtained in this way were crossed with one another. Two, ht_6 and ht_9, showed

TABLE 2

Number of mutant plaques in stocks and plaques of h^+ parents

Source	Parent genotype	Total no. plaques	No. mutants
Stock	Wild	10000	2
Stock	r_1	5000	10[1]
Plaque 3	r_{13}	22000	7
Plaque 4	r_{13}	10000	5
Plaque 5	r_{13}	10000	4
Plaque 6	r_{13}	8000	1
Plaque 1	r_1ht_2	10000	2
Plaque 3	r_1ht_2	10000	4
Plaque 5	r_1ht_2	8500	3
Stock	$r_1ht_2ht_3$	31000	15
		124500	53

[1] Only one of these mutants has been studied so far.

Stocks or plaque suspensions were diluted to give 6–800 plaques per plate plated with the sensitive cell S. The plates were replicated to the indicator cell B/2. The mutant plaques gave very turbid replications as compared to no replication from the wild plaques. The mutant plaques, after identification by comparison of the replica and master plates, were picked from the S plate to be used in subsequent crosses.

very little or no recombination with each other (less than 0.1 percent). The other mutants showed recombination with one another and with ht_6 and ht_9. In view of the intracluster distances found by DOERMANN and HILL (1952) and BENZER (1955), lack of recombination may reflect only our present level of resolution.

At present, mutants at 12 or 13 loci have been isolated. Eight were isolated in the above experiment. Three, ht_2, ht_3, and ht_4, were segregated from the variant M6. Finally, ht_1 and ht_5 were segregated from T2*h'* and from Hershey's T2*m*, respectively. From the high mutation rate and from the number of loci now identified, it is concluded that the phenotype of host-range is under the control of many different loci.

Mapping of the ht *loci*

There was no evidence of selection against any of the parental or recombinant types. The possibility of selection was tested in crosses between h^+ and *ht* parents marked with different alleles of *r* where it was found that the input and output ratios of the *r* markers agreed within experimental error and that the differences between frequencies of reciprocal recombinants were not statistically significant.

In crosses involving phages differing in two *ht* loci, (fig. 6), the double recombinant was usually counted and often picked for use in subsequent crosses. However, the degree of turbidity is far more susceptible to variations in plating than is the absence of replication of the wild type. Therefore, the percentage recombination was uniformly determined by doubling the frequency of the wild recombinant. The results of all crosses used in this study are presented in table 3. The observed recombination frequency is given with the recombination frequency corrected as described in footnotes of the table so as to show the extent of the correction. A map of recombination

TABLE 3

Recombination frequencies of the ht *loci*

Cross	No. plaques examined	% recomb. obs.	% recomb. corrected	Avg. % recomb.	S.D.
$h' \times h^+$	301	2.3	4.6		
	1391	1.7	4.3		
	1692			4.4	±1.3
$h' \times ht_2$	589	0.8	1.0		
$ht_2 \times r_1$	389	34.4	42.0		
$ht_2 \times r_7$	502	18.5	22.6		
$ht_2 \times r_{13}$	906	1.2	1.7		
	540	0.9	1.1		
	1446			1.5	±0.3
$ht_2 \times ht_3$	389	16.5	20.1		
	668	19.9	23.1		
	1057			22.0	±1.9
$ht_2 \times ht_4$	2277	24.2	34.0		
	1367	23.1	37.9		
	3644			37.2	±1.9
$ht_2 \times ht_6$	665	18.6	24.8		
	296	25.0	28.8		
	961			26.0	±1.8
$ht_2 \times ht_7$	910	35.4	41.2		
$ht_2 \times ht_9$	645	17.7	26.8		
	929	12.1	14.9		
	1574			19.8	±5.8
$ht_2 \times ht_{10}$	314	35.2	44.0		
$ht_2 \times ht_{11}$	297	32.3	38.4		
	130	29.0	34.5		
	427			37.2	±1.8
$ht_2 \times ht_{13}$	545	21.3	28.8		
$ht_3 \times r_1$	388	38.1	50.0		
	560	41.4	49.3		
	948			49.6	±0.3
$ht_3 \times r_2$	655	26.0	30.2		
	729	27.7	33.4		
	1384			31.9	±1.6

TABLE 3. *Continued*

Cross	No. plaques examined	% recomb. obs.	% recomb. corrected	Avg. % recomb.	S.D.
$ht_3 \times r_7$	710	16.3	20.1		
	259	21.2	29.9		
	690	24.1	28.0		
	655	26.1	31.4		
	2314			26.8	±4.6
$ht_3 \times r_{13}$	928	16.7	21.1		
	1305	12.8	20.6		
	2233			20.8	±0.3
$ht_3 \times h$	463	16.4	21.3		
	373	14.5	18.8		
	159	15.1	19.4		
				20.1	±1.2
$ht_3 \times ht_4$	530	37.0	48.7		
$ht_3 \times ht_6$	900	12.7	14.4		
	647	10.0	13.7		
	1547			14.1	±0.4
$ht_3 \times ht_7$	495	33.1	41.4		
	875	29.5	36.9		
	1370			38.6	±2.2
$ht_3 \times ht_8$	545	38.9	49.2		
$ht_3 \times ht_9$	208	11.1	12.8		
$ht_3 \times ht_{10}$	591	28.1	33.5		
$ht_3 \times ht_{13}$	388	32.5	42.2		
	739	37.6	44.2		
	1127			43.5	±1.0
$ht_4 \times r_1$	562	29.9	36.2		
	1137	32.0	39.5		
	1699			38.4	±1.6
$ht_4 \times r_7$	188	38.3	49.7		
$ht_4 \times h$	215	30.7	36.5		
	849	33.9	41.2		
	1064			40.4	±1.9
$ht_4 \times ht_5$	445	9.9	11.6		
	483	7.9	10.3		
	928			10.9	±0.7

TABLE 3. *Continued*

Cross	No. plaques examined	% recomb. obs.	% recomb. corrected	Avg. % recomb.	S.D.
$ht_4 \times ht_6$	740	38.6	43.9		
	721	26.1	34.8		
	418	42.6	52.3		
	1879			42.3	±6.7
$ht_4 \times ht_7$	793	0.8	0.9		
	696	0.6	0.7		
	1169	0.8	1.0		
	2658			0.9	±0.06
$ht_4 \times ht_8$	501	25.1	29.9		
$ht_4 \times ht_{12}$	920	6.7	8.6		
$ht_4 \times ht_{13}$	531	13.6	15.6		
	578	15.7	18.9		
	1109			17.3	±1.7
$ht_5 \times ht_6$	559	39.7	46.2		
$ht_5 \times ht_7$	290	6.9	8.2		
$ht_5 \times ht_8$	560	20.0	25.0		
$ht_5 \times ht_{10}$	577	2.8	3.5		
$ht_5 \times ht_{12}$	387	13.8	17.9		
$ht_5 \times ht_{13}$	268	8.2	10.6		
	415	9.2	10.7		
	683			10.7	±0.5
$ht_5 \times ht_1$	772	1.0	1.3		
	607	1.3	1.7		
	1379			1.5	±0.2
$ht_6 \times r_1$	358	24.3	28.3		
	440	30.0	34.1		
	798			31.5	±2.9
$ht_6 \times r_7$	815	29.9	40.5		
$ht_6 \times r_{13}$	373	29.8	34.6		
$ht_6 \times ht_7$	301	37.9	44.6		
	796	40.4	48.7		
	477	34.4	40.5		
	2000			46.2	±3.5
$ht_6 \times ht_8$	671	31.3	37.7		
	307	31.9	37.5		
	978			37.6	±0.05

TABLE 3. *Continued*

Cross	No. plaques examined	% recomb. obs.	% recomb. corrected	Avg. % recomb.	S.D.
$ht_6 \times ht_9$	664	0	0.2		
	787	0.5?	0.6?		
	1451			questionable	
$ht_6 \times ht_{10}$	261	39.1	46.5		
$ht_6 \times ht_{12}$	574	32.1	36.5		
$ht_6 \times ht_{13}$	276	34.1	38.8		
	467	30.8	39.0		
	871	35.4	40.2		
	649	37.0	44.0		
	2263			40.1	±2.3
$ht_7 \times r_1$	266	33.1	40.8		
$ht_7 \times r_2$	746	37.9	43.6		
$ht_7 \times r_{13}$	1064	36.1	46.5		
$ht_7 \times ht_8$	658	17.6	21.5		
	859	14.7	20.4		
	1517			20.9	±0.6
$ht_7 \times ht_{12}$	821	6.8	8.5		
	488	8.6	10.2		
	1309			9.2	±2.2
$ht_7 \times ht_{13}$	388	21.1	24.8		
$ht_8 \times ht_{12}$	636	17.9	21.1		
$ht_8 \times r_2$	538	41.8	43.9		
$ht_{10} \times ht_{11}$	1193	1.6	2.0		
$ht_{10} \times ht_{12}$	652	19.6	24.5		
$ht_{12} \times r_1$	564	16.8	23.7		
	849	23.6	29.5		
	1413			27.2	±3.0
$ht_{12} \times h$	849	33.9	42.4		

The number of plaques is the total number of progeny plaques counted and classified as to phenotype.

The observed recombination frequency in the case of crosses between two *ht* parents was calculated by dividing twice the number of wild recombinants by the number of progeny sampled. In the case of crosses between *ht* and *r* parents, the number of both recombinants was divided by the total number of progeny counted.

The corrected recombination frequency is the observed recombination frequency corrected according to LENNOX *et al.* 1953, which adjusts for progeny produced by cells receiving exclusively or predominantly either one of the two parents.

Average recombination frequency is the average of all crosses between any two loci weighed in reference to the sample size of each cross.

frequencies showing the linear relations of the *ht* loci (fig. 1) has been prepared from table 3. This map can be converted to a linkage map by correcting back to the first round of mating as discussed by DOERMANN and HILL (1953), the reviews of DOERMANN (1953), HERSHEY (1953) and VISCONTI and DELBRÜCK (1953).

The criterion for the unlinked condition is a consistently high recombination frequency between all members of a group of linked markers with all members of another group of linked markers. In general, the values used for the unlinked condition were the recombination frequencies between markers on the second linkage group and those on the combined first and third linkage groups. From 53 crosses involving 32,070 plaques, the average recombination value for the unlinked condition, corrected for the multiplicity of infection and parental ratio (see table 3), was 42.4 percent with a standard deviation of 5.0. When the multiplicity is further corrected for the discrepancy in cell killing as affected by cell size, (DULBECCO 1949), the average recombination value approaches 45 percent.

The linear order of the loci has been established by a series of crosses between the different loci within a linked region. No discrepancy in the order as presented (fig. 1) was found except in the comparison of very long with very short distances. A large number of crosses was needed to establish the discreteness and order of the 13 widely scattered markers; and experiments which were considered to be largely qualitative are included in table 3. A more complete analysis of the linked regions is now being undertaken, particularly in reference to additivity.

The *ht* loci are scattered on linkage groups II and III. They are not obviously clustered as are the *r* markers in T2 and the *r* and *tu* markers in T4. However, eight of the thirteen lie in groups of two or more, linked within two percent of one another. The loci ht_6 and ht_9 have not been definitely separated from each other by recombination (fig. 1). This distribution is considered to be a tendency toward clustering.

The marker ht_{13} is located on the third linkage group. It shows ten percent recombination with ht_5 which is closely linked (1.5 percent) to m_1. However ht_{13} gave an average recombination of 27.2 percent with r_1 which is on the first linkage group.

TABLE 4

Recombination frequencies showing linkage between groups I and III

Loci	Exp. no.	Total no. plaques	No. recomb. plaques	% recomb. corrected	Recomb. avg. %
r_1 to h	1	473	196	49.3	
	2	355	139	51.3	
	3	433	163	47.6	
					49.3 ± 2.8
h to ht_{13}	1	473	196	49.3	
	2	355	123	42.8	
					46.5 ± 6.4
ht_{13} to r_1	1	473	142	35.7	
	2	355	88	30.6	
					33.5 ± 5.0
ht_6 to h	3	433	94	27.4	
ht_6 to r_1	3	433	164	47.8	

Since this value lies just outside the range for unlinked factors, two three-factor crosses were done involving r_1, ht_{13}, and *h*, which is unlinked to both other markers. All categories of recombinants can be scored in this cross. The results are given in table 4. The percent recombination between the unlinked factors, r_1 with *h*, and *h* with ht_{13}, are significantly higher than the recombination of ht_{13} with r_1. The linkage of r_1 with ht_{13} joins linkage groups I and III. The locus ht_6 which is on linkage group II shows a recombination frequency with r_1 (31.5) which is lower than the range for unlinked factors. However, in this case the three-factor cross involving *h* showed clearly that ht_6 was unlinked to r_1.

DISCUSSION

It is evident from the work presented here that host-range, like other phenotypes in the phages, is under the control of many loci. The *ht* loci are scattered throughout the genome and extend over 190 recombination units. Of the first 13 isolated, 12 are separable from one another and give little evidence of the strong clustering phenomenon found in the *r* and *tu* markers of the T-even phages.

The *ht* loci, as noted by HERSHEY and DAVIDSON (1951), and as clearly shown in this work are additive. A dosage effect can be observed as the number of *ht* modifications within single particles increases. FRASER and DULBECCO (1953) reported a series of host cells and mutant phages in T3 which closely parallel the series described here. They explained their results on the additivity of phenotypic expression of different loci. The additive effect may be the rule among repetitive loci affecting host range. DOERMANN and HILL (1951) found a similar additive effect of the *tu* mutants of T4. Nothing strictly comparable has been observed with the *r* mutants.

From the work of HERSHEY and DAVIDSON (1951) and from current experiments in our laboratory, it is known that adsorption is involved, at least in part, in the host-range phenotype as affected by the *ht* loci. Phages containing a single mutant *ht* locus adsorb much more slowly to B/2 than do phages containing double or multiple doses of mutant loci some of which adsorb at a rate comparable to that of *h* under the conditions of the experiment. The wild type shows no adsorption to B/2. Thus, many genetic loci control the same physiological character and the effect of the different loci is additive in respect to that character. Adsorption, which depends on the protein portion of the phage particle, could be increased by a single chemical change which could occur at multiple sites in the protein. However, adsorption could also be affected by any number of different changes in the protein and, as in the case of the *r* phenotype, the *ht* loci may eventually be differentiated by the manner in which they produce their phenotype. It is possible that each locus affects a different change, any one of which is adequate to give some adsorption, and the different ones augment one another. It is evident that the control of adsorption by the *ht* loci is different from the control of adsorption by the different alleles of the *h* locus which appear to act in an all-or-none manner.

STREISINGER using another system (T2L, B, and B2L) has shown that the *h* region, covering an area of two recombination units, is a very dense cluster of mutational units which can be differentiated from one another by recombination. This system allows for the detection of very low recombination values because of

the tremendous difference in adsorption between particles in the wild and in the *h* condition. It may be that the *h* region in T2H is also a cluster of very tightly linked sub-loci which cannot be detected by our present experimental methods. Two of the *ht* loci isolated from M6 are within two percent of the *h* locus. HERSHEY and DAVIDSON (1951) isolated a turbid mutant which showed no recombination with *h*. The relatively high frequency of clear mutants associated with the *h* locus can be explained if one assumes a large group of tightly linked loci. Such a group would act as a functional unit and might therefore be expected to act in an all-or-none manner.

In a very broad sense repetitive genes in phages are analogous to the polygenes postulated to explain inheritance of quantitative characters in higher organisms. The effect of mutation at individual loci is small. However, the effects are cumulative which would give a strong selective value to particles containing several *ht* mutations under the proper environmental circumstances. Incidentally there would be a selective advantage towards clustering which would lead to stability during the recombination process (MATHER 1940).

The polygenic control of host range greatly enhances the potential number of host-range variants in a viral population. Where multiple infection of a host cell is possible, recombination between mutants will create strong host-range variants. These facts may have eventual application to epidemiology.

The linkage groups I and III of T2 have been combined through ht_{13} and r_1. STREISINGER (personal communication) has shown a similar linkage involving the same region in T4. He also has evidence of a weak linkage between groups I and II in both T2 and T4. The reduction of the linkage groups to one in these phages is more compatible with current conceptual schemes of replication and mating.

SUMMARY

The host-range phenotype, like other phenotypes of the coliphage T2, is controlled by many repetitive loci scattered throughout the genome. In the coliphage T2 thirteen loci (ht_1-ht_{13}) have been isolated and located in relation to each other and in relation to previously mapped markers. Mutations at any one of the *ht* loci produces the same phenotype. When mutants are accumulated by recombination within the same phage their effects are additive. The different alleles of the locus *h* determine the base host-range to which mutations at the different *ht* loci also add. The properties of repetitive loci are compared to those of polygenes of higher organisms.

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GENETIC VARIANCE OF RANDOM-INBRED LINES OF DROSOPHILA MELANOGASTER IN RELATION TO COEFFICIENTS OF INBREEDING

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THE chief genetic effects of inbreeding is to decrease heterozygosity and the extent to which this happens depends on how close is the relationship between the mated individuals. WRIGHT (1921a) has been able to calculate theoretically the rate at which heterozygosity is reduced. Therefore, it should be possible to calculate how many generations must elapse before the same degree of homozygosity is achieved under different systems of mating. Thus, in the absence of selection, fifty percent of the original heterozygous loci will be homozygous in the third and the eighth generations in matings between brother-sister and double-first cousins respectively.

Genetic variance of a given character is reduced by inbreeding because of the increase of homozygosity within inbred lines. Therefore, animal breeders correct the estimates of heritability, i.e. of genetic variance, derived from more or less inbred populations, to give a better estimate in non-inbred populations as shown by LERNER (1950).

The validity of such forecasts depends on:

1. Equal survival ability of homozygotes and heterozygotes; if the latter are favoured by natural selection, then the actual increase in homozygosity will be less than expected.

2. Negligible frequency of mutations which increase the variability during inbreeding.

3. Recognition that adjustment of heritability estimates based on inbred populations is based upon the assumption that the genetic variation responsible for the observed heritability is strictly additive in nature.

WRIGHT (1920, 1934a) analyzed the total variance of white spotting and of polydactyly respectively in guinea pigs, inbred by brother-sister matings for more than twenty generations. He found that the genetic variability of both characters was almost eliminated in the highly inbred lines. MACARTHUR (1944, 1949) reported changes in heritability in lines of mice selected for twenty-one generations, with an average rate of decrease of one to three percent of heterozygosity per generation. Although the heritability was initially 25 percent, it declined to 10 percent in the later generations. It is not possible to determine to what extent selection and inbreeding were responsible for this change.

TANTAWY (1952) and TANTAWY and REEVE (1956) inbred lines of *Drosophila melanogaster* with different systems of mating. Estimating the heritability of the body size at the same level of homozygosity they found that such estimates differed significantly in different inbred lines. The heritability of wing length declined almost to the theoretical expectation, at the higher levels of homozygosity in the case of brother-sister matings, and showed an insignificant decline with milder rates

of inbreeding. The same phenomenon has been noticed in different selected lines inbred at different rates, as shown by TANTAWY (1954, 1956a, 1956b) who reported that heterozygosity declined in the inbred selected lines with sibmatings, but not in other selected inbred lines of double-first cousin matings.

The present experiment was designed to provide more information on whether the genetic variance of body size of *Drosophila melanogaster*, estimated from wing and thorax length, declines under random mating according to the theoretical expectation at different levels of homozygosity. It is also intended to study the effects of various rates of inbreeding, giving the same or nearly the same coefficient of inbreeding, on different characters.

TECHNICAL PROCEDURE

Since it is necessary to relate the heritability estimates in any inbred line to that observed in the random mating foundation population, two estimates for the heritability of wing and thorax length have been carried out in the initial stock. The stock used in the present study is the Crianlarich stock of *Drosophila melanogaster* which has been kept by mass mating in populations of about 20 pairs. Wing and thorax length were measured by an instrument devised by ROBERTSON and REEVE (1952a). The mean wing and thorax lengths in this stock with their respective coefficients of variation have been reported by TANTAWY (1956b).

Two systems of mating have been used in different inbred lines, i.e. brother-sister and double-first cousins. These systems have been discussed in detail by TANTAWY (1956b). In each system of mating five parallel inbred lines have been carried. The periodic estimates of the heritability in the various inbred lines within each system of mating have been averaged to provide a measure of the change in the heritability at particular levels of inbreeding. Extensive records for numbers of eggs laid and numbers of adults hatched in each vial have been collected.

Estimation of heritability for initial random mating foundation population

The heritability was estimated in the initial foundation population by two progeny tests, one with random mating and the other with assortative mating. In the former, eggs were collected in five vials, and from each vial 20 pairs of adult flies (20 males and 20 virgin females) were measured and then mated at random in pairs, giving altogether 100 pair-matings. After the parents were kept together in well yeasted vials for three days the eggs were collected from each pair over four successive days and cultured in the usual way. From each of the cultures set up on the first two days, five pairs of flies were measured on each day to provide an estimate of the average size of the progeny of each mating.

In case of assortative matings, the procedure was exactly the same in each line as in the case of random mating except that mated individuals were selected on the basis of their wing length. Five pairs were selected for large size, and another five pairs were selected for small size, and in each case matings were in pairs, i.e. largest $\times$ largest, second largest $\times$ second largest and so on, smallest $\times$ smallest, second smallest $\times$ second smallest and so on.

Estimation of heritability for random inbred lines

Brother-sister matings: Five inbred lines mated at random were maintained i.e. A, B, C, D and E. At every particular generation, i.e. at a known rate of inbreeding of 25, 50 and 75% coefficients of inbreeding, heritability estimates were carried out in the following way.

From each of the five parallel inbred lines two families were maintained and from each family five pairs of flies (males and virgin females) were measured and mated assortatively. Matings secured from the ten families were fed for three days and then transferred to oviposition vials for the collection of eggs which were introduced into cultures over four successive days. Five females from among the adult flies hatching in each culture on the first two days were measured.

Double-first cousin matings: Five parallel random mated inbred lines i.e. A′, B′, C′, D′ and E′ have been set up. The general procedure for the heritability estimates is the same as in the case of brother-sister, except that the relationship between the parents bred in the assortative matings is different. The relationship between the mated individuals is of double-first cousins.

Effects of inbreeding

From each of the five parallel inbred lines, at the same or nearly the same coefficients of inbreeding, within each system of mating, five random mated pairs were taken, and the eggs were collected in well yeasted vials for four successive days. Ten pairs of flies were measured from the first random mating on each of the first two days, giving altogether 100 pairs. Such samples of flies were measured at about 25, 50 and 75% of inbreeding.

At all levels of inbreeding a control stock was maintained by mass mating. Eggs were collected in four vials on the same four days on which the eggs in the inbred lines were collected. From each vial five pairs of flies were measured from each mating on the first two days, giving 40 pairs.

Care was taken to treat uniformly the different inbred lines and the control stock. The parents of all pair matings in the different inbred lines within each system were mated exactly at the same age and were handled under similar environmental conditions.

Statistical procedure

Since each progeny test maintained at any given coefficient of inbreeding for the estimation of the genetic variance depended on the regression of the female progeny only on the average size of the mid-parents, the results should be corrected for the absence of the male measurements. Heritability estimates obtained from different progeny tests have been corrected by an equation worked out by the application of the path coefficient method, as shown in diagram 1.

By the application of the path coefficient method devised by WRIGHT (1920, 1921a, 1921b, 1934b) to diagram 1, an equation can be worked out to correct the heritability estimates based on the regression of the female offspring on the mid-parent size in the absence of male measurements.

From diagram 1 it can be seen that

$$r_{PP'} = \tfrac{1}{2}h^2$$

and since, $\bar{P}'$ is completely determined by P' and P' therefore, $2X^2 = 1$ if there is no correlation between mates, and

$$X = \sqrt{\frac{1}{2}}$$

From the same diagram we can find that:

$$r_{P\bar{P}'} = 2Xr_{PP'} = \frac{2}{\sqrt{2}} \times \frac{1}{2}h^2 = \frac{1}{\sqrt{2}}h^2$$

$$\therefore \quad b_{P\bar{P}'} = \frac{\text{Cov.}\,(P\bar{P}')}{\sigma^2 P'} = \frac{\text{Cov.}\,(P\bar{P}')}{\sigma P \sigma \bar{P}'} \times \frac{\sigma P}{\sigma \bar{P}'} = r_{P\bar{P}'}\frac{\sigma P}{\sigma P'}$$

$$\therefore \quad b_{P_\varphi} \text{ on } \bar{P}' = \frac{1}{\sqrt{\frac{1}{2}}}h^2 \times \frac{\sigma P_\varphi}{\sigma \bar{P}'}$$

and

$$\sigma^2 \bar{P}' = \frac{1}{4}(\sigma^2 \text{ males} + \sigma^2 \text{ females})$$

$$= \frac{1}{2}\left(\frac{\sigma^2 \text{ males} + \sigma^2 \text{ females}}{2}\right)$$

and, if mating is at random, $\sigma P_\varphi = \sigma P'_\varphi$

$$\therefore \quad b_{P_\varphi} \text{ on } \bar{P}' = \frac{1}{\sqrt{2}}h^2 \times \frac{\sigma P'_\varphi}{\frac{1}{\sqrt{\frac{1}{2}}}\sqrt{\frac{\sigma^2 \text{ males} + \sigma^2 \text{ females}}{2}}}$$

$$= h^2 \times \frac{\sigma P'_\varphi}{\sqrt{\text{mean variances of the two sexes}}}$$

$$\therefore \quad \text{corrected heritability} = b_{P_\varphi} \text{ on } \bar{P}' \times \frac{\sqrt{\text{mean variances of males and females}}}{\text{standard deviation of females}}$$

i.e.,

$$h^2 = b_{P\bar{P}'}\frac{\sqrt{\frac{1}{2}(\sigma^2 P_\varphi + \sigma^2 P_\sigma)}}{\sigma P}$$

As an example of the application of this equation, table 1 shows the results of a progeny test where the heritability estimate is based on the progeny of both sexes and on the female progeny only before and after correction.

Table 1 shows that the heritability estimates based on the regression of female progeny on mid-parent, although not significant, is lower than that of the regression of average progeny size (both sexes). But this difference between the two estimates is eliminated when the first estimate is corrected for the absence of male measurements by the application of the previously given equation.

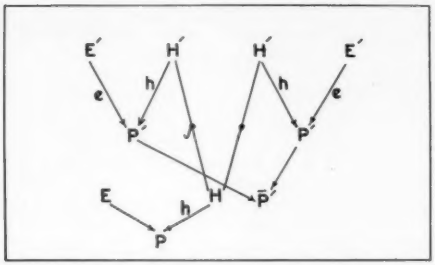

DIAGRAM 1.—Illustrates the relationship between female offspring (P) and the mid-parent size ($\bar{P}'$), where P' and P' are the parents and H' and H' are the heredity of the parents and H is the heredity of the offspring.

TABLE 1

Comparison of heritability estimates of wing length in the initial stock. Calculations based on the regression of the offspring size (sexes averaged), on the regression of the female offspring size only, and the heritability corrected for the absence of male measurements. These estimates are also corrected for the magnified variance between the parents

Sexes averaged	Female progeny	Corrected heritability
41.12 ± 10.20	36.91 ± 12.31	42.61 ± 8.52

RESULTS

Heritability of wing and thorax length and the genetic correlation between them in the initial foundation population

Two progeny tests have been carried out in the initial stock to estimate the heritability of wing and thorax lengths, using the regression method described by LUSH (1949). One of these estimates involved random matings, and the other assortative matings, the latter estimate being corrected for the magnified variance between the parents as suggested by REEVE (1953). The results are presented in table 2.

The weighted means of the heritability estimates for both the characters indicate a high genetic variance due to the additive gene effects.

TANTAWY (1956b) reported that the genetic variance of the same stock as used in another experiment was about 45 percent for wing length and 47 percent for thorax length. These estimates are ostensibly higher than the average estimates given for both characters in table 2. About one year intervened between the two experiments, and it appears that the heritability in the random mated foundation population may have declined, probably due to loss of genetic variability.

Heritability of wing and thorax length in *Drosophila melanogaster* of different stocks has been estimated by ROBERTSON and REEVE (1952a), REEVE and ROBERTSON (1953), TANTAWY (1956a, 1956b) and TANTAWY and REEVE (1956). All estimates of the heritability of wing and thorax length range between 30 percent and 45 percent.

Genetic correlation between wing and thorax length was estimated with the aid

TABLE 2

Heritability of wing and thorax length and the genetic correlation between them in the initial foundation population

Type of mating	Wing length %	Thorax length %	Degrees of freedom	Genetic correlation %
Random	37.22 ± 7.13	36.95 ± 7.37	86	73.87
Assortative	41.12 ± 10.20	39.95 ± 12.00	38	74.83
Weighted means	38.51 ± 4.30	37.77 ± 4.80	124	74.35

of HAZEL's formulae (1943) in the first test, and by REEVE's formulae (1953) in the second test. After correction for the magnified variance between the parents in the assortative matings, the average genetic correlation was found to be 75.35 percent, which shows a decline also from what had been obtained by TANTAWY (1956b) for the same stock.

The Nettlebed stock of *Drosophila melanogaster* showed, as reported by REEVE and ROBERTSON (1953) and TANTAWY (1952 and 1956a), an average genetic correlation of 65–75 percent between wing and thorax length, which is very close to that obtained for the present stock.

Heritability estimates in the inbred lines

Heritability estimates of wing and thorax length and the genetic correlation between them could be changed either by environmental modification, by intensive inbreeding without selection as shown by TANTAWY (1952) and TANTAWY, REEVE and ROBERTSON (1956), or by intensive inbreeding with selection as reported by TANTAWY (1956a, 1956b). Continued selection without inbreeding is one of the major factors that could change these genetic parameters as reported by REEVE (1950), REEVE and ROBERTSON (1953) and ROBERTSON and REEVE (1952a) working on body size of Drosophila, and ALAN ROBERTSON (1954) working on the chaeta number.

Heritability estimates for wing length derived by various progeny tests in the present study (corrected for the absence of male measurements) at any given coefficient of inbreeding in both systems of mating are presented in figure 1 with their respective standard errors as plotted in the inset graph of figure 1. Expected heritability estimates for the genetic variance initially available in the foundation population at different levels of inbreeding are calculated according to LERNER (1950).

It can be seen from figure 1 that progeny tests carried out at 25 percent coefficient of inbreeding in both systems of mating give values for the heritability estimates above the average of the initial population, which support the view that this stock has lost some genetic variance.

In both systems of mating heritability estimates, at 25 percent of inbreeding, are higher than the theoretical expectation, and at 50 percent of inbreeding both systems do not show any reduction in the heritability estimates from that found in the initial population. But at higher rates of inbreeding, i.e. 75 percent, there is no significant difference between the theoretical expectation and the observed values in the case of brother-sister matings. At the same level of homozygosity in case of the milder

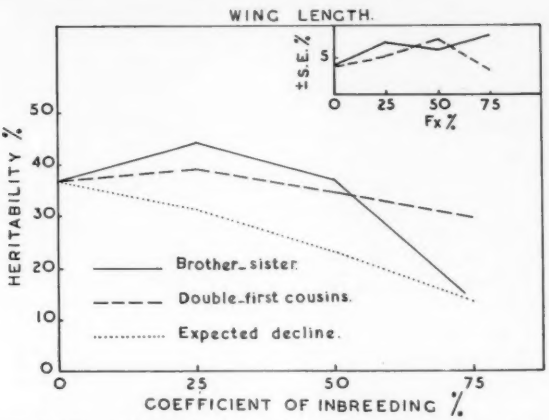

FIGURE 1.—Heritability estimates of wing length in different systems of mating and the expected decline at different coefficients of inbreeding, with their respective standard errors (S.E.%).

forms of inbreeding, i.e. double-first cousins, heritability estimates show little decline from the initial value and are definitely higher than the theoretical expectation.

Thorax length (data not included) behaves almost identically with wing length; the heritability estimates show reduction at the lower levels of inbreeding in neither system. At higher rates of inbreeding, genetic variance of thorax length has declined almost to the theoretical values in case of brother-sister matings.

Since the average heritabilities at 25 percent and 50 percent coefficients of inbreeding have not declined, it appears that inbreeding made no significant differences in the genetic variance between the initial stock and the different inbred lines. But with further inbreeding, there is a definite decline in the genetic variance almost to the theoretical expectation in case of brother-sister matings, and no decline in case of milder forms of inbreeding.

Genetic correlation between wing and thorax length

The two progeny tests on the initial population provide data for the calculation of this genetic correlation. The average genetic correlation between wing and thorax length was found to be 74.35 percent (table 2) in the initial stock. Genetic correlations in the different inbred strains, at different levels of inbreeding, are shown in figure 2.

It can be seen from figure 2 that the genetic correlation between wing and thorax length, in case of sib matings, shows no decline in lines with up to 50 percent of inbreeding, while at higher levels there is a tendency for this correlation to decrease. In the case of double-first cousin matings, such genetic correlation remains almost constant without change at all known rates of inbreeding.

As the genetic variability is reduced, it would be expected that the genetic correlation should be reduced also. This could be noticed in case of the higher levels of in-

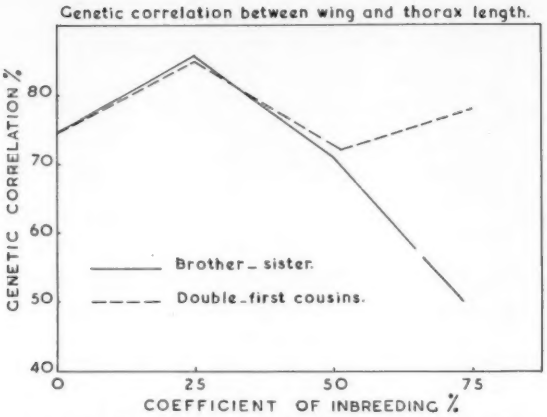

FIGURE 2.—Genetic correlation between wing and thorax length in the different inbred lines in relation to coefficient of inbreeding.

tensive inbreeding, where the genetic variability declines almost to the theoretical expectation.

From these results, it can be concluded that genes affecting both wing and thorax length, particularly in the case of milder rates of inbreeding, are still segregating; this gives further evidence of considerable genetic variability.

Effects of inbreeding

The depression effects of inbreeding may be due to: a. Appearance of some deleterious recessive genes in homozygous conditions, which would otherwise have remained undetected in heterozygous conditions, hidden by their dominant alleles; b. Fixation of an allele at a locus where the heterozygous condition is favoured, i.e. elimination of overdominance.

The present study may throw some light on the origin of the depression effects of inbreeding in relation to different levels achieved by various systems of mating. Effects of inbreeding on different characters will be discussed for similar levels of homozygosity. These characters are wing and thorax length, and percentage of hatchability of eggs.

The data concerning the effects of inbreeding on wing and thorax length are given in the form of averages for all flies, both sexes, measured at a particular level of inbreeding. The results for wing length in both systems of mating are shown in figure 3.

Figure 3 shows clearly that different rates of inbreeding have no depressive effects on the wing length with both systems of mating at the lower levels of inbreeding, i.e. 25 percent. But at 50 percent coefficient of inbreeding, matings between brothers and sisters cause a depressive effect of about 2.77 percent below the control level, after which the wing length stabilizes at this level. Matings between double cousins show a less deleterious effect of inbreeding; these inbred lines show an average decline of 1.78 percent below the control level at 50 percent coefficient of inbreeding.

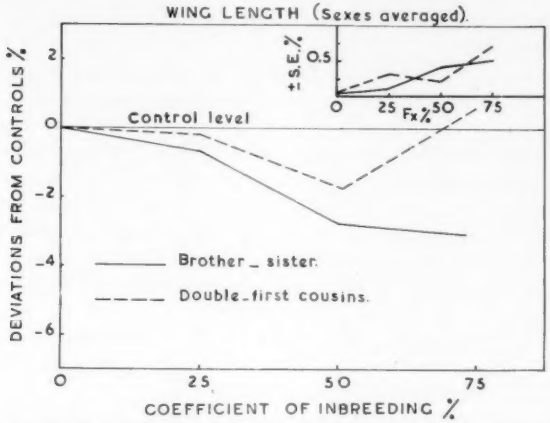

FIGURE 3.—Effects of inbreeding on wing length in relation to the level of homozygosity. Results are presented as percentage deviations from the control level, with their respective standard errors (S.E.%).

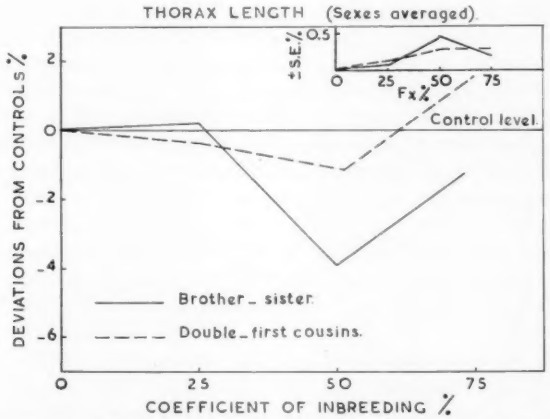

FIGURE 4.—Effects of different intensities of inbreeding on thorax length in relation to the level of homozygosity. Results are presented as percentage deviations from the control stock, with their respective standard errors (S.E.%).

At higher rates of inbreeding, there is no significant difference between these inbred lines and the control level.

Brother-sister matings without selection from a random mated population produced decline in wing length as reported by ROBERTSON and REEVE (1952, 1955a, 1955b) and ROBERTSON (1954). Intensive random inbreeding with brother-sister matings on strains of Drosophila selected for long wings maintained by outbreeding

system, showed a decline in wing length in the data of TANTAWY (1953) and REEVE and ROBERTSON (1953a).

In case of thorax length, figure 4 represents the effects of different intensities of inbreeding on this character. It can be seen from figure 4 that inbreeding has no effect at the level of 25 percent. At the level of 50 percent, brother-sister matings show a decline of 3.96 percent below the control level, while at the same level double-first cousin matings show a decline of only 1.60 percent below the control level. At higher rates of inbreeding the thorax length behaves in the same way as wing length in double-first cousin matings. But in sib matings thorax length increases towards the control level, which shows a contrast to what was noticed for wing length. These different effects might be due to some unknown environmental factors which affect wing and thorax for the control stock differently.

Phenotypic variations of wing and thorax length are measured by coefficients of variation and the results are presented in figures 5 and 6 for wing and thorax length respectively with their respective standard errors.

The average level of the control stock is shown in figures 5 and 6. This level does not show any marked change from generation to generation. These figures indicate that, at the lower levels of inbreeding i.e. 25 percent and 50 percent coefficients of inbreeding, there is no significant difference between the variability of the various inbred lines, and the control stock. But at higher levels of inbreeding wing length shows a decline in its phenotypic variability in both systems of matings. Lines inbred by brother-sister matings show a more marked decline than in the case of matings between double-first cousins. These results agree with those reported by ROBERTSON and REEVE (1952a, 1952b) working on body size of Drosophila, and RASMUSON (1952) working on bristle numbers, who showed that inbreeding with brother-sister matings causes a marked decline in the coefficients of variation of the characters under consideration.

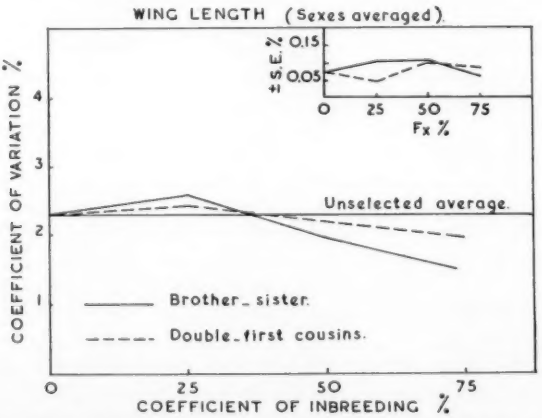

FIGURE 5.—Coefficient of variation of wing length in the different inbred lines, with their respective standard errors (S.E.%).

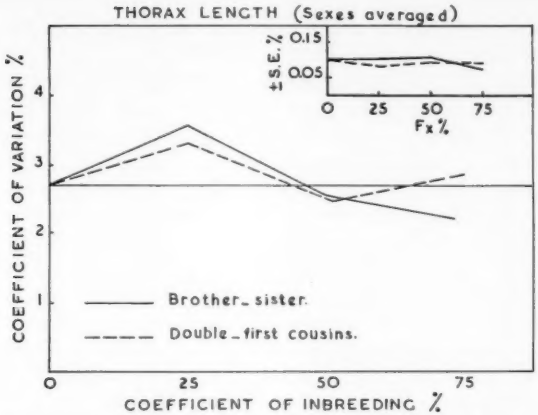

FIGURE 6.—Coefficient of variation of thorax length in the different inbred lines, with their respective standard errors (S.E.%).

Thorax length shows no decline in its variability in the latter system while it declines below the control level in the former one. It could be noted from these results that brother-sister matings show a relatively greater reduction in the coefficients of variation than matings between double-first cousins, particularly at the higher levels of homozygosity.

There is an apparent agreement between the decline in the coefficients of variation and the reduction of the genetic variation at the higher levels of inbreeding in sib matings. This is an expected result, since phenotypic variation is due to mixture of genetic environmental effects. If the genetic variance declines with the increase of homozygosity within inbred lines, phenotypic variation will also decline.

Percentage emergence is calculated as the number of adults expressed in percentage of the number of eggs deposited in each vial for each mating on four successive days. Figure 7 represents the percentage emergence in all different inbred lines compared with the average unselected stock.

The control level is shown only in figure 7 since the control stock showed no marked decline from the initial starting point. Percentage emergence at 25 percent of inbreeding in the case of double-first cousin matings is not shown since the experiment failed because of some environmental fluctuations.

Figure 7 clearly demonstrates that inbreeding at the lower levels gives little depression effects on the percentage of emergence with either system of mating. This percentage declines from 50 percent of inbreeding in both systems, and the differences between the percentages of emergences in the two systems remain constant, being about five percent at each level of inbreeding. At each coefficient of inbreeding brother-sister matings show the most decline in percentage emergence.

Inbreeding experiments on Drosophila have shown reduction in fertility of the inbred lines, as shown by TANTAWY (1956a), TANTAWY and REEVE (1956), SMITH *et al.* (1955) and HOLLINGSWORTH *et al.* (1955).

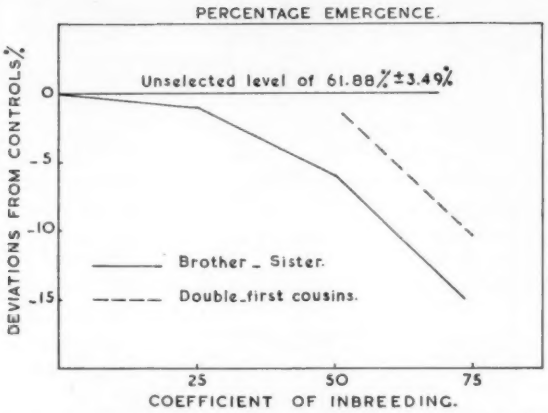

FIGURE 7.—Percentage emergence in the different inbred lines compared with the control level. Data are presented as percentage deviations from the controls.

DISCUSSION AND CONCLUSIONS

The present experiment was designed to study the effects of different intensities of inbreeding on the genetic variability and its relation to the theoretical expected decline. Although the effects of inbreeding are widely known, there is very little information about the reduction of the genetic variance, i.e. of the heritability, of a given character in relation to the level of homozygosity attained with different systems of mating. TANTAWY (1952) and TANTAWY and REEVE (1956) found that the heritability of the body size in Drosophila in inbred lines with different rates of inbreeding declines more slowly than does the expected reduction in homozygosity.

The frequent use of coefficient of inbreeding to estimate the genetic variability presupposes that the gene differences concerned are largely additive in action and that natural selection does not favour heterozygosity compared with homozygotes or *vice versa*. It is feasible to test the validity of this assumption only in suitable experimental animals.

In the present experiment two different characters in a non-inbred population of *Drosophila melanogaster*, namely wing and thorax length, were chosen to study the fraction of the total variation in each character which is due to heredity. The genetic variances of both characters are represented as the weighted means of two progeny tests which have been carried out in the initial foundation population. These estimates are found to be 38.51 percent and 37.77 percent for wing and thorax length respectively. These estimates measure the additive gene effects but may include a small portion of epistatic variation and additional genetic variation due to the effects of dominance and epistasis.

Judging from the effects of long inbreeding on the variance of body size at least 50 percent of the variance in the unselected stock is environmental, as shown by ROBERTSON and REEVE (1952b). One could estimate for wing length that 30 percent is additively genetic and 20 percent due to non-additive genetic effects.

In the present investigation the heritability of wing and thorax length in lines inbred at different rates but supposedly to the same levels of homozygosity was estimated. It can be seen that the expected and the observed heritabilities are in agreement at the higher levels of inbreeding by brother-sister matings. But on comparing the lines inbred at different rates no differences were found in the earlier stages of inbreeding nor in the later stages of matings between double-first cousins. These results do not show a uniform tendency for the loss of heterozygosity to be proportional to the rate of inbreeding, but they do suggest that brother-sister matings are more effective than slower rates of inbreeding in eliminating heterozygosity. This conclusion agrees well with the results reported by TANTAWY (1956a, 1956b) on different selected inbred lines, and with those reported by TANTAWY and REEVE (1956) on different random inbred lines. There must be some natural selection for heterozygosis with characters such as body size. These results support the views of THOMPSON and REES (1956) who stated that during inbreeding, natural selection would favour to some extent the more heterozygous genotype. They also reported that selection operates in favour not of heterozygotes in general but of particular heterozygous combination.

The present experiment was carried out under optimal conditions to minimize the effects of environmental fluctuations. If environmental conditions were different, selection intensities would be different also. It is possible that if the experiment were repeated under severe conditions like starvation or overcrowding, natural selection of heterozygotes would be more clearly demonstrated.

Results secured from studying other characters agree in showing that heterozygosity may persist in the inbred lines. Estimation of the genetic correlation between wing and thorax length clearly demonstrates that genes responsible for body size are segregating, and this is further evidence of considerable genetic variability especially in the lines inbred with the milder rates of inbreeding. Body size and its phenotypic variability showed most reduction in the more intensely inbred lines which support the views that the genetic variability has declined more in the case of brother-sister matings.

Percentage emergence shows that the effects of inbreeding on viability are more drastic for sib matings than the milder rates of inbreeding, particularly at the higher levels of inbreeding. This is probably because the recessive genes become homozygous at a faster rate in the case of sib matings. A mutation which affects the eye colour appeared in one of the inbred lines of sib matings at 50 percent coefficient of inbreeding. This is recessive, single gene mutation, and it is under investigation. The different results obtained with fertility agree with those reported by different workers in showing that there is a positive relation between percentage emergence and increase of homozygosity by inbreeding.

The previous results suggest that:

a. The effects of inbreeding may be influenced by the genetic constitution of the foundation population, the rate of inbreeding and possibly also by the environmental conditions which will influence the intensity of natural selection for a particular genotype.

b. Natural selection may reduce the rate of increase of homozygosity below that

expected in any system of mating at the lower levels of inbreeding. But at higher levels, brother-sister matings appear to be more effective in eliminating heterozygosity for a character such as body size than less intensive systems of inbreeding.

One may suggest from these results that such a situation may hold true in the case of livestock, where the rate of inbreeding is generally very low, and computed coefficients of inbreeding may overestimate the real homozygosity attained in the inbred lines. Animal breeders make allowances for the amount of inbreeding in their herds when they estimate the expected heritability of a particular character in the non-inbred stock. This danger of bias has been postulated on theoretical grounds, though no evidence has been obtained for it except by TANTAWY (1956a, 1956b) on selected inbred lines and by TANTAWY (1952) and TANTAWY and REEVE (1956) on random inbred lines of *Drosophila melanogaster*.

WRIGHT and EATON (1929) stated that "The unavoidable natural selection of more vigorous lines might keep a greater amount of heterozygosis in minor factors in the stock than that expected by theory." SCHOFFNER and SLOAN (1948) who adjusted the heritability estimates for different characters in poultry for the amount of inbreeding of their flock stated that "If the reduction in the genetic variability is actually less than the inbreeding coefficient implied then there would tend to be an exaggeration of these estimates which are higher."

SUMMARY

1. *Drosophila melanogaster* (Crianlarich stock) has served as material for the present study.

2. Two characters are chosen for estimation of the heritability, namely the wing and the thorax lengths. These characters are correlated with body size. The weighted means of the two progeny tests indicate that the heritabilities of these characters are 38.51 ± 4.30 and 37.77 ± 4.80 respectively in the initial foundation population.

3. Inbreeding was carried out by means of two different systems of mating, i.e. brother-sister and double-first cousin matings. The heritability of wing and thorax length was estimated by progeny tests carried out at the coefficients of inbreeding of 25%, 50%, and 75%. Heritability estimates for wing length remain almost constant at the lower levels of inbreeding, and decline almost to the theoretical expectation in the case of brother-sister matings at the higher levels. Matings between double-first cousins cause a slight reduction at any given coefficient of inbreeding from the initial population.

4. There is a positive genetic correlation between the wing and thorax lengths in the initial population. It remains nearly stable at the lower levels of inbreeding in both systems of mating, but at the higher levels this genetic correlation declines only in the case of brother-sister mating, and remains constant in the other system.

5. Body size and its phenotypic variability declines in all systems of mating, especially at higher levels of inbreeding; the matings between sibs show the highest reduction.

6. Percentage emergence has decreased in the inbred lines at every level of homozygosity. The sib matings show the greatest reduction.

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INBREEDING DEGENERATION IN TETRAHYMENA[1]

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INBREEDING degeneration has been observed in many higher plants and animals and its physical basis and its bearing on the genetic economy of these organisms have been widely discussed (GOWEN 1952; LERNER 1954). While some genetic problems such as gene action, mutagenesis and genetic recombination have been actively and profitably pursued with microorganisms, relatively little work on inbreeding degeneration and its converse, hybrid vigor, has been undertaken with the smaller and more readily studied forms. The chief reason for this neglect is that most microorganisms are haploid for a major portion of their life cycles, and both heterosis and inbreeding degeneration are characteristic of diploids. Among the haploids only the inbreeding effects in yeast (WINGE and LAUSTSEN 1940) and heterocaryosis in ascomycetes (DODGE 1942; BEADLE and COONRADT 1944) bear a similarity to such phenomena in higher organisms and these similarities may be superficial. Even the organisms with predominantly diploid life cycles often undergo autogamy or some other form of intensive inbreeding which assures a high degree of homozygosity and for this reason have an "essentially haploid" economy free from the disadvantages of inbreeding and from certain of the advantages of crossbreeding observed in other diploids.

Many, but not all, of the Ciliated Protozoa do, however, show a type of inbreeding degeneration similar to that in higher organisms (JENNINGS 1944a, 1944b). Recently work on *Tetrahymena pyriformis* has demonstrated that this Ciliate has a genetic system essentially like that in other crossbreeding diploids and that it may provide a useful model system for studying the effects of inbreeding. It undergoes conjugation, but autogamy has not been observed; it shows deterioration upon inbreeding and vigor is restored on outcrossing. The occurrence of inbreeding degeneration is not, of course, an unmixed blessing and presents problems for a genetic program in which homozygosis is a valuable tool. Fortunately, strains can be obtained which survive the various inbreeding crises and return to approximately the state of vitality observed in the original crossbred strains.

The Ciliated Protozoa in general are useful in a comparative study of the types of genetic economy since closely related forms may show great differences in their breeding patterns and genetic structure (SONNEBORN 1956). The observations to be reported here have been acquired incidentally in the course of studying other traits in Tetrahymena and do not constitute a definitive analysis. A thorough evaluation of the phenomena reported must await more extensive study.

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MATERIALS AND METHODS

Inbreeding effects were first observed in *T. pyriformis* during attempts to establish reasonably homozygous strains from two wild cultures of variety 1, WH-6 (mating type I) and WH-14 (mating type II), supplied by ELLIOTT. The cross between these strains yields few viable progeny which have completed the conjugation process, but these few can be crossed and yield progeny with good viability and a total of seven different mating types (NANNEY and CAUGHEY 1953). Two different inbred series have been developed which differ in the mating types produced at conjugation; "Family A" produces types I, II, III, V and VI in characteristic frequencies and "Family B" regularly yields types II, III, IV, V, VI and VII. Genetic studies (NANNEY, CAUGHEY and TEFANKJIAN 1955) show that the differences in mating type potentialities in these families are controlled by a single pair of genes; Family A is homozygous for a gene $mt^{(IV,\ VII)-}$ and Family B is homozygous for an allele $mt^{(I)-}$. Within a family the mating types are distributed more or less at random among the caryonides (first fission products, each with a single new macronucleus) after conjugation so that more often than not a single exconjugant yields two mating types (NANNEY 1956). Since these caryonides have the same genetic constitutions but different mating types, close inbreeding, equivalent to selfing in other forms, can be carried out. The cytological events occurring at conjugation (ELLIOTT and HAYES 1953; NANNEY 1953; RAY 1956) and the general methods used in these studies (NANNEY and CAUGHEY 1955; NANNEY, CAUGHEY and TEFANKJIAN 1955) have been described previously and will not be presented here.

TYPES OF INBREEDING ANOMALIES

In establishing the inbred strains by crosses of sister caryonides, inbreeding degeneration became apparent after three or four generations and took a number of forms which need to be distinguished.

Death at conjugation

In most of the crosses to be reported the exconjugants and the first fission products (caryonides) were isolated into separate containers and were allowed to give rise to cultures. In some instances the exconjugants undergo no fissions, however, and the first fission products cannot be separated; in other cases the exconjugants divide a few times and then die. In general the pairs can be separated into two classes—those whose progeny fail to give rise to cultures in the first depression slide and those whose progeny grow indefinitely. Occasionally one of the exconjugants or one of the caryonides fails to give rise to a culture, but a high intrapair correlation is observed and many of the exceptions are certainly due to injury of the cells during isolation. The class "dead at conjugation" is made up of those pairs, none of whose progeny undergoes ten fissions.

Nonconjugation

When normal conjugation occurs and new macronuclei are formed, the progeny show a period of sexual immaturity lasting usually about sixty fissions. Moreover, new mating types arise and these are distributed approximately at random among

the caryonides. Some of the pairs in certain crosses, however, separate and yield cultures only of the two parental types (one parental type from one mate and the other parental type from the other mate) and without an immature period. These pairs have been interpreted as pairs in which the normal reorganization process was incomplete and in which new macronuclei did not develop. The nonconjugant category, therefore, includes pairs showing cytological irregularities during conjugation. Careful cytological examinations of many crosses showing nonconjugation have not yet been carried out and different kinds of cytological irregularities may emerge when this is done.

One may not conclude, however, that all "nonconjugation" is due to cytological aberrations. If pairs are isolated shortly after conjugation is initiated in a mixed culture, a large fraction will separate without even initiating a reorganization. Even when pairs are isolated several hours after the beginning of mating, a small percentage will be of this type, for pairs are formed continuously in a mating mixture. These nonconjugant pairs can often be distinguished from those undergoing a partial reorganization since the separated cells undergo fission without the lag normally accompanying conjugation. No such distinction is made, however, in the following tabulations; pairs were generally isolated about twelve hours after the initiation of conjugation when the latter type of nonconjugants should be infrequent.

Delayed micronuclear anomalies

A third type of anomaly (in some ways the most interesting) has a late onset, rarely being manifested earlier than the 40th postzygotic fission and occasionally not appearing until after 100 or more fissions. Clones showing this aberration are designated as "semi-amicronucleate" (S.A.) for reasons to be described shortly. The first visible sign of this condition is the appearance, at first in low frequency, of small rounded crinkled cells lying at the bottom of a depression culture as it begins to starve. The frequency of these cells rises gradually in subsequent transfer cultures until nearly all the cells are of this type. When the crinkled cells are isolated they are incapable of initiating a new culture and the clones can be maintained in serial single cell transfers only through isolations of the apparently normal cells; selection of normal cells does not, however, result in the establishment of normal cultures. Since in our work the isolations are usually made when the cells have not yet starved and when the two types of cell are difficult to distinguish, the S.A. cultures are not maintained for many transfers.

A cytological examination of these cultures reveals that nearly all of the visibly abnormal cells lack micronuclei and that most of the normal cells contain at least one and often several micronuclei; hence, the term "semi-amicronucleate" for the cultures. Even when micronuclei are present, however, they may be grossly abnormal; some are many times as large as normal micronuclei and others may be as small as 10 percent of the normal size. The facts that the amicronucleate cells never give rise to cultures and that cells with any of several anomalous micronuclear conditions can give rise to cultures suggest that the micronuclei in these strains are necessary for continued growth, but that the precise content of the micronucleus may not be important in this respect.

The derivation of the micronuclear anomalies was studied by examining stained

preparations of the S.A. cultures during their growth period. The chief aberration (loss of micronuclei from some cells) appears to result from a failure to coordinate the divisions of the micronucleus with the divisions of the cell. In normal cells the micronucleus migrates to a mid-point in the cell and has completed its division by the time the cell begins to constrict. In S.A. cultures dividing cells may be found in which the micronucleus has either remained near one end of the cell and has not divided, or has begun to divide in an excentric position so that the entire spindle or a large portion of it lies within one of the presumptive daughter cells. Thus, following cell division one of the daughter cells is amicronucleate. The origins of the minute micronuclei and of the very large micronuclei have not yet been clearly established.

Although continued growth is not possible in the absence of a micronucleus, the stained preparations include dividing cells in which no micronuclei are apparent. Hence, amicronucleate cells are capable of undergoing at least one cell division before they die.

When the S.A. cultures are sexually mature they may be crossed with either normal cultures or other S.A. cultures showing a different mating type. A cytological examination of conjugation under these circumstances demonstrates two things. First, at least some of the amicronucleate cells can pair with cells of another mating type, and in mixtures of two S.A. cultures pairs may be found in which both members are amicronucleate. These cells are, however, the phenotypically normal amicronucleates and may have been recently derived; the grossly abnormal crinkled cells have not been observed to form pairs. Secondly, even those cells with micronuclei usually do not behave properly in conjugation. As in cell division, many of the micronuclei fail to make the important nuclear migrations and conjugation is halted at intermediate stages. In any event, conjugation involving an S.A. culture has always led to death. This fact must be qualified slightly. On a few occasions crosses have been made with clones which were "presumptive" S.A. clones, i.e. clones in which the S.A. condition appeared later, and from these crosses viable exconjugants were obtained. Some of these surviving exconjugants, moreover, appeared to be free from the S.A. stigma in further growth. These facts suggest that the establishment of a new nuclear constitution is in some instances capable of rescuing otherwise doomed clones, but that this rescue must occur before the clone has expressed the first signs of deterioration. Unfortunately, little information is available about this aspect of the problem since sexual maturity and the S.A. condition usually have their onset at about the same time.

The evidence that the S.A. condition has a genetic basis comes from a consideration of its distribution among related clones. In a given cross all the progeny of some pairs will be normal and all the progeny of other pairs will become S.A. When observations are discontinued at 100 fissions, as in most of the experiments to be reported, a few pairs will include progeny of both types, but these may be interpreted as S.A. pairs in which the S.A. condition had not yet appeared in some cultures at the time observations were stopped. This interpretation was examined more critically for one cross by expanding the progeny of a number of pairs until each pair was represented by 16 cultures, all of which were maintained in daily isolations until they had undergone 150 fissions. By 100 fissions most of the pairs gave either all or

no S.A. cultures and by 120 fissions complete concordance was observed. Hence, the S.A. condition is a "pair" characteristic and is probably determined by events occurring at conjugation, most reasonably by the establishment of a genetic constitution at that time.

A condition similar to that described above may also be observed under certain other circumstances, but the causal factors are clearly different. When normal clones are kept under continued growth for very long periods of time (500 to 1500 fissions) changes occur similar to the aging effects reported in other Ciliates (See SONNEBORN 1954). Among these changes are micronuclear anomalies resulting again in S.A. cultures. No confusion with these cultures is possible in the experiments reported since the observations were discontinued long before the aging effects become apparent, but the existence of this other kind of S.A. culture suggests that what is being studied may be some kind of premature aging effect.

THE COURSE OF INBREEDING DEGENERATION

Although the various signs of inbreeding deterioration appeared during the initial breeding experiments, useful quantitative data are not available for this period; proper screening techniques for nonconjugants had not yet been adopted and the S.A. condition was not always recognized. The information to be presented, therefore, concerns the events occurring after an intercross between the two inbred families. A fourth generation Family A caryonide was crossed with a sister caryonide from the same family to give an F_5; similarly a third generation Family B caryonide was crossed with a sister caryonide to yield an F_4. (It should be pointed out, perhaps, that these Family A and Family B clones were not chosen at random but were selected on the basis of their ability to produce a reasonably high frequency of viable progeny.) One of the Family A caryonides was then crossed with one of the Family B caryonides to yield the intercross generation (I_1) from which all the subsequent cultures were derived. The results of these first crosses are given in table 1.

The method of scoring the frequencies of the anomalies requires some comment. First, the frequency of the S.A. cultures is meaningful only in terms of the total pairs which could have shown this condition, i.e., those pairs which were maintained long enough to manifest the trait; this automatically excludes both the pairs that died and the pairs that failed to complete conjugation and were discarded. This treatment permits the frequency of S.A. pairs to vary from 0 to 100 percent, re-

TABLE 1

Initial crosses in the inbreeding experiments

Parents	Total pairs	Normal	Died	Viable	Non-conjugant	Viable conjugants	S.A.
Fam. A; $F_4 \times F_4$ (F_5) A	45 (100%)	20 (45%)	15 (33%)	30 (100%)	3 (10%)	27 (100%)	7 (30%)
Fam. B; $F_3 \times F_3$ (F_4) B	45 (100%)	20 (45%)	16 (36%)	29 (100%)	5 (17%)	24 (100%)	4 (17%)
Fam. A $F_4 \times$ Fam. B F_3 (I_1)	59 (100%)	57 (97%)	0 (0%)	59 (100%)	2 (3%)	57 (100%)	0 (0%)

gardless of the fraction of the pairs which died at conjugation or were nonconjugant. Similarly, the test for nonconjugation can be applied only to those pairs which survive long enough to be examined and the frequency of nonconjugants is expressed as a fraction of the viable pairs. The frequency of dead conjugants and the frequency of normal conjugants are expressed as fractions of the total pairs isolated.

These results show a fairly high incidence of anomalies in both the inbred strains when crossed among themselves. Less than half the pairs isolated gave rise to normal cultures. Secondly they show the elimination of these anomalies in the intercross. Except for a few pairs in the nonconjugant class, which may have been pairs which were disturbed in the early stages of conjugation, all the pairs gave rise to normal cultures. This high viability is not permanent, however, and gradually disappears when inbreeding is resumed.

The general features of the inbreeding effects can be demonstrated by a presentation of the history of one inbred series. The upper graph in figure 1 depicts the frequencies of pairs which failed to survive conjugation in successive generations of crosses between sister caryonides. In the first and second intercross generations no death was observed, but the two separate crosses made for the I_3 generation showed 8 percent and 36 percent respectively. Progeny from the more viable of these crosses were selected and four different I_4 crosses were made; all yielded some inviable progeny with frequencies ranging from 11 percent to 92 percent. Although some viable progeny were produced in each of these crosses, many of the survivors were either nonconjugant or S.A. For the I_5 generation one cross was made using a pair from the most viable of the previous crosses; 28 percent of these pairs failed to survive. In the I_6 generation two crosses were made, one of which yielded 40 percent death at conjugation and one of which showed no death, the first instance in

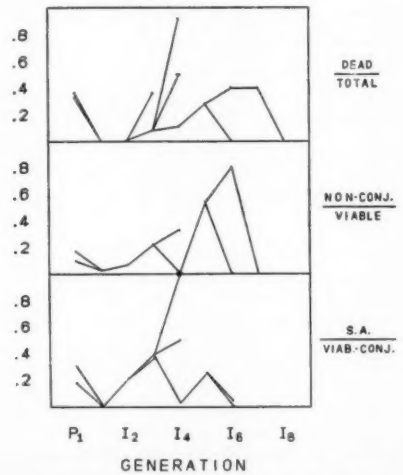

FIGURE 1.—The course of inbreeding degeneration and recovery in strains of *Tetrahymena pyriformis* initiated from moderately inbred parents (P_1) and inbred through crosses of selected sister caryonides for eight generations.

which viability improved. At this point the selection procedure was altered and the I_7 was derived from the less viable cross in the previous generation; again 40 percent inviability was obtained. Finally, a single I_8 cross was made and all the pairs survived.

The frequencies of non-conjugating pairs in this same series are given in the middle graph in figure 1 and show the same general trends. The inbreeding effect becomes progressively more noticeable for several generations and then begins to disappear. Similarly, the frequency of the S.A. condition (lower graph, figure 1) rises with inbreeding, but with selection for normal parents gradually decreases until it has been entirely lost. Hence, all three indices of inbreeding degeneration rise in time and are then progressively reduced until, after 6 to 8 generations of selfing with constant selection, strains are obtained which are approximately as free from defects as the I_1 generation.

Two other inbred series of about the same size have been studied and with approximately the same results. The series differ in the times at which the inbreeding crises became most acute and in the relative and absolute contributions of the different kinds of anomalies, but all three show a peak for each kind of abnormality followed by a dropping off. A detailed analysis of these data is not indicated since great variability is encountered in the various subseries and sufficient subseries were not studied. Moreover, larger samples would be required for the individual crosses; most of the qualitative conclusions drawn are based on sample sizes of only about 30 pairs. It is reasonably clear, however, that the various kinds of degeneration are at least partially independent of each other; any one may be prevalent in a cross in which the others are negligible.

THE PHYSICAL BASIS OF INBREEDING EFFECTS

Two general explanations of inbreeding deterioration may be considered in light of these observations. One explanation holds that the deleterious effects of inbreeding are due to the exposure of harmful recessive genes normally carried as heterozygotes in a crossbreeding population; according to this interpretation a population should upon inbreeding pass through a series of crises as the recessive genes are brought into homozygous condition and eliminated. After this "purification" procedure the strains should return to normal if a strict enough selection can be practiced to prevent the fixation of deleterious genes. A second type of explanation attributes heterosis primarily to heterozygosity accompanied by overdominance; the heterozygote is superior to either of the homozygotes. This interpretation leads to the expectation of deterioration upon inbreeding but does not predict a return to normal vitality unless some special mechanism is evolved to maintain heterozygosity. When a return to normal vitality is not observed, distinctions between the two hypotheses are difficult, but when vitality is restored after intensive inbreeding the evidence strongly supports the first hypothesis. The observations on Tetrahymena suggest that at least for the traits studied overdominance is not a major factor. Perhaps when other characteristics of the inbred lines are studied (such as growth rate or resistance to harmful environmental agents) this conclusion will have to be modified.

Although an explanation based on harmful recessive genes explains the observa-

tions in a general way, efforts to detect genes with individually significant effects have been unsuccessful. If many of the signs of degeneration were due to individual genes, the greatest deterioration would be expected in the I_2. The I_1 generation would be the most heterozygous of the generations and each lethal gene should be homozygous in one fourth of the I_2; if two lethals were present seven sixteenths of the I_2 should be affected, etc. With selection against these homozygotes the frequencies of abnormal pairs should decrease upon further inbreeding. Yet none of the indices reached 25 percent in the I_2 and all continued to rise for several more generations. Hence, the most reasonable explanation for these effects is the accumulation in homozygous condition of many genes (polygenes) individually insignificant, but in the aggregate (perhaps after reaching a threshold level) capable of severely damaging the cell.

The available data do not permit a precise evaluation of the number of such genes, but suggest a large number. The relative independence of the various kinds of abnormalities suggests at least three different groups of genes concerned with different kinds of cellular functions, and the number of genes in each group must be fairly large. This is shown best, perhaps, by examining the results of crosses within and between strains in an intermediate stage of inbreeding. Five different sets of sister caryonides in the I_2 generation were chosen for such an analysis; all were selected to be homozygous for the $mt^{(IV,\ VII)-}$ allele and on the basis of progeny tests which showed about the same level of S.A. pairs among their progeny. *1a* and *1b* were derived from a single I_1 pair; *2* was derived from a different I_1 pair and *3a* and *3b* came from a third. Each of these sets was crossed with itself and also with each of the others and all the indices of degeneration were calculated. Only those for the S.A. characters will be given since they illustrate the kinds of results

TABLE 2

The frequency of the S.A. condition among the progeny of crosses of selected I_2 parents. 1a and 1b are sets of sister caryonides derived from the same I_1 cross; 2 is from a second I_1 cross and 3a and 3b are from a third

Parents	S.A. progeny	Pairs examined
1a × 1a	.39	50
1b × 1b	.36	50
2 × 2	.23	50
3a × 3a	.30	50
3b × 3b	.51	50
1a × 1b	.36	50
1a × 2	.00	30
1a × 3a	.00	50
1a × 3b	.04	80
1b × 2	.36	80
1b × 3a	.08	50
1b × 3b	.04	30
2 × 3a	.07	30
2 × 3b	.07	30
3a × 3b	.43	50

observed (table 2). When crossed with themselves each set yields a moderate frequency of S.A. progeny—from 23 percent to 51 percent. Most of the intercrosses yield a lower frequency than either parent when crossed with itself, suggesting that the two parents differ in the factors responsible for the trait. Some exceptions to this rule are observed, however, for *1a* and *1b* yield about the same frequency of S.A. pairs when intercrossed as when selfed. Similarly *3a* and *3b* yield about the same results when intercrossed as when selfed. *1a* and *1b* can be distinguished, however, on the basis of crosses to *2*; *1a* in this cross produced no S.A. cultures and *1b* produced 36 percent. Hence, among these five sets of I_2 strains, showing similar rates of S.A. production when selfed, at least four different genetic constitutions can be distinguished for the S.A. trait alone. Since other selfing crosses yield all intermediates from 0 to 100 percent S.A. progeny, a large number of genotypes and hence genes must be expected.

SUMMARY AND CONCLUSIONS

The information presented demonstrated that variety 1 of *Tetrahymena pyriformis* possesses the type of genetic economy usually associated with crossbreeding diploids. During intensive inbreeding various signs of inbreeding degeneration appear; these signs include death at conjugation, failure to complete nuclear reorganization, and delayed micronuclear anomalies. Each of these kinds of aberration rises gradually for several generations, but with constant selection for normal progeny, strains may be derived which have passed each of the crises and which are apparently as vigorous as the original crossbred strains. A preliminary genetic analysis suggests that the anomalies are due to the accumulation in homozygous condition of combinations of genes which individually have little or no detectable effect.

These observations by no means settle all the questions relating to the breeding system of these strains in nature. In particular they do not explain the high incidence of death and nonconjugation encountered in crosses taken from the wild. A possible explanation may however be attempted. JENNINGS (1944a) observed that clones of *Paramecium bursaria* underwent a regular sequence of changes during their life cycles. Following conjugation they entered a period of sexual immaturity lasting for a variable, but usually long, period of time. Following this period the cultures became capable of mating and usually bred true for a particular mating type. If crosses were made between two young clones, they often resulted in many viable progeny, but if the same cross was repeated from time to time as the cultures grew older, the frequency of the viable progeny dropped gradually to zero. SONNEBORN (1954) observed similar aging effects with clones of *Paramecium aurelia* and further discovered that gross chromosomal rearrangements accompanied the aging processes. Hence, it is possible that the F_1 mortality observed in many crosses with Tetrahymena is due to the use of aged clones in which chromosomal aberrations have been established. According to this interpretation the initial death at conjugation is due to age-correlated chromosomal aberrations acting as dominant lethals. In any case, it is certainly clear that chromosomal rearrangements are common in some of the stocks that yield inviable progeny (RAY 1955) and that Tetrahymena stocks can become senile in the laboratory (ALLEN and NANNEY unpublished).

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THE EFFECTS OF COMBINED RADIATIONS ON CROSSING OVER IN DROSOPHILA MELANOGASTER[1]

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IN attempts at an experimental approach to the problem of the mechanism of crossing over many modifying agents have been used: temperature (PLOUGH 1917), aging (BERGNER 1928), inversions (STEINBERG 1936), mineral content (BROWNING 1949), and radiation (MULLER 1925). As diverse as are these agents, they all have one effect in common. They modify the rate of crossing over in either the region of the kinetochore or the region very distal to it. In most cases there is observed a pattern of change which KIKKAWA (1934) called, "the proximal increase and compensatory distal decrease". This similarity of effect suggests a common path of action by all the agents; but few attempts to construct a unifying hypothesis have been presented. Three which deserve mention are: the mechanical hypothesis of KIKKAWA (1934), one involving coiling modifications by MATSUURA (1940), and one stressing the role of heterochromatic areas (SCHULTZ AND REDFIELD 1951). These have much in common, and offer a good starting point for a generalized hypothesis.

In recent experimental investigations, much emphasis has been laid upon the effect of radiations. WHITTINGHILL and his associates (WHITTINGHILL 1954; HINTON and WHITTINGHILL 1950) have done a thorough analysis of this aspect; but they have devoted most of their discussion to a consideration of the time at which crossing over occurs rather than the mechanism whereby the radiation affects the process. However, since much stress is put upon the role of recombination in the gonial cells, one has the feeling that these authors believe that the radiation acts to cause increase in the number of breakage points. Such a role of modifying agents is also apparent in the recent publication of LEVINE (1955), in which it is suggested that the correlation between mineral deficiency and crossing over increase can be explained by rupture of the chromosomal strands at points of calcium binding. Both agents (X-ray and mineral deficiency) are known to disintegrate the chromosome structure (STEFFENSEN 1955) and both increase crossing over in certain regions. Although there are a number of objections to this idea, it is a natural assumption and one which merits further investigation. It was the increased breakage hypothesis which these experiments were designed to test.

It has been demonstrated that following treatment with infrared radiation chromosomes of Drosophila are broken more frequently by exposure to X-rays (KAUFMANN, HOLLAENDER and GAY 1946). It was felt that this potentiating effect of the infrared might apply to a normal chromosome breakage process in the course of crossing over. A test of this idea was made with negative results (IVES, FENTON, YOST and LEVINE 1953), and it was concluded that the type of recombination

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exhibited by crossing over is quite different from that shown by radiation induced translocations. However, this does not apply to the idea that radiation increases crossing over by an increase in breakage. It was felt that although there is no increase in recombination with infrared alone (just as there is no increase in chromosome breakage with infrared alone), there might be a potentiating effect of the infrared when used as a combined radiation. If this proved true, it should then be possible to test this effect with other modifying agents and perhaps gain some knowledge of the suggested common pathway of action. Furthermore, a recent paper by SNOAD (1955), has indicated that infrared radiation modifies the number of chiasmata in Tradescantia. This made an investigation of the effect of infrared on crossing over imperative.

Recently acquired evidence that the effects of infrared treatment in promoting chromosome breakage in Tradescantia are more pronounced with X-radiation than with gamma radiation (SWANSON, personal communication) suggests that the breakage of chromosomes by infrared radiation is related to the ion density of the supplementary radiation. It seemed possible that the use of different ionizing radiations might facilitate interpretation of the data. If the results were to show a greater effect in the combined X-ray-infrared series than in the combined gamma ray-infrared series, they might indicate similar mechanism to be operating in the induction of crossing over and in chromosome breakage. For this reason the tests were made using both types of radiation.

MATERIALS AND METHODS

The stocks used in this experiment were an Oregon-R wild type, a second chromosome marker stock, *net b cn bw*, and a first chromosome marker stock, *y* cl^6 ras^2 *f*. The wild type stock had been inbred for 130 generations by single pair brother-sister matings. The marker stocks were synthesized in this laboratory about 15 years ago and had been maintained in close-bred vial cultures with about ten pairs of parents in each generation. At the beginning of the study, each stock was shifted to half pint culture bottles and maintained thereafter with about 100 pairs of parents per generation.

Females tested for crossing over in these studies were heterozygous for the wild type and one or other mutant marker stock. They were backcrossed to the marker stock males immediately after treatment. Individual females were mated to two or more males in shell vials of corn meal-molasses-agar-yeast medium and were shaken to new shell vials every day in most cases. In one series, the flies were shaken every three days. The treated virgins were collected at one time, meaning that their age did not vary by more than ten hours; they were exposed to infrared radiation at approximately 24 hours of age. All cultures were raised at 25°C.

The infrared source and the method of exposure has been described previously (YOUNG, YOST, IVES and LEVINE 1953). After a 24 hour exposure to infrared, at 10°C, the flies were treated with either X or gamma rays. The X-rays were delivered from a General Electric industrial machine, operated at 80 kv and 10 ma. The gamma radiation was obtained from a 440 curie Co^{60} slug. In both cases the total dose was 1400r, as measured with an appropriate Victoreen dosimeter. The intensity was

200r per minute in both cases. At all times, the control flies received exactly the same treatment as the experimental flies, with the exception of exposure to the radiation.

Analysis of the data was done by using the chi-square test. The data are lumped only where it is permissible. Analysis of reciprocal classes showed no significant differences in these classes. There was no indication of clusters of crossover types.

EXPERIMENTAL RESULTS

For the sake of brevity certain abbreviations have been used in the tables. They are as follows: C—control flies; X—X-rayed flies; γ—gamma rayed flies; IR+—flies exposed to infrared radiation as a pretreatment to the ionizing radiation; (vs C) the chi-square test is computed against the control values; (vs X)—the chi-square test is computed against the values from the X-rayed series.

Table 1 presents the data obtained when the flies were exposed to infrared and X-rays. In this series the flies were shaken every three days, the marker stock was *net b cn bw*. Since the regions *net-b* and *cn-bw* are so large, the data for crossing over in these cases are omitted. There was no significant change from normal (in either direction) shown by these regions. It is evident that only the heterochromatic region near the kinetochore is being tested by this stock. These data indicate that from about the fourth day on both X-rays and combined treatment increase the rate of crossing over near the kinetochore. However, there is no indication of an added

TABLE 1

Crossing over in young adult females exposed to infrared radiation and X-rays. Dose: 24 hrs. IR; 1400r

Day	Treatment	No. flies	% crov. *b-cn*	χ^2 (vs C)	χ^2 (vs X)
1–3	C	797	4.0		
	X	1223	3.7	0.1636	
	IR + X	1240	4.8	0.5922	1.8028
4–6	C	1022	2.7		
	X	1774	4.7	6.7768***	
	IR + X	1739	5.2	9.2476***	0.3492
7–9	C	1041	3.1		
	X	1610	9.0	35.6986***	
	IR + X	1529	8.5	30.6969***	0.2427
10–12	C	1295	3.7		
	X	1520	8.3	25.4081***	
	IR + X	1739	7.9	22.6094***	0.1810
13–15	C	1227	5.1		
	X	1470	8.0	9.1764***	
	IR + X	1646	7.6	5.1324***	0.1512
16–18	C	1051	5.3		
	X	1217	6.5	1.3797	
	IR + X	1348	7.9	6.3415**	1.9677
19–61	C	939	6.1		
	X	975	8.7	4.9091*	
	IR + X	1082	7.9	2.7300	0.4093

* Significant at the 0.05 level.

** Significant at the 0.02 level.

*** Significant at the 0.01 level.

effect of the infrared pretreatment. It was felt that the three day laying period might obscure the effects of the infrared treatment, since it was of only 24 hours duration, and it is known that some agents show effects for only as long as the duration of the treatment (PLOUGH 1917). To test this a new series was set up with transfer of flies every day. These data are presented in table 2. In this table data from two runs are presented. Because of the inherent variation in the data it was felt that separate presentation of the results would be better. It can be seen that with only one exception the values tend to be higher for the treated series than the controls from the first day on. It can also be seen that from the fourth day on the results are completely consistent. However, there is no significant difference between the X-ray and the combined treatment which is consistent. Thus the combined

TABLE 2

Crossing over in young adult females exposed to infrared radiation and X-rays. Dose: 24 hrs. IR; 1400r

		Run 1				Run 2			
Day	Treatment	No. flies	% crov. *b-cn*	χ^2 (vs C)	χ^2 (vs X)	No. flies	% crov. *b-cn*	χ^2 (vs C)	χ^2 (vs X)
1	C	454	4.8			262	3.4		
	X	322	6.5	0.9738		281	4.3	0.0324	
	IR + X	357	6.7	1.3512	0.0148	294	7.1	3.6789	2.1630
2	C	433	4.2			415	3.6		
	X	553	5.8	1.3681		400	2.5	0.8733	
	IR + X	567	5.8	1.3681	0.8729	377	5.0	0.9658	3.4427
3	C	502	4.0			391	3.1		
	X	525	4.0	—		383	6.3	9.5454***	
	IR + X	490	5.3	0.9338	0.9728	407	3.4	0.7059	8.1262***
4	C	549	5.3			432	3.7		
	X	651	6.8	1.1377		362	7.5	5.4280**	
	IR + X	528	8.3	4.0511*	1.0053	431	4.9	0.7059	2.3250
5	C	509	4.5			353	6.0		
	X	568	8.8	7.7969***		354	8.9	1.3774	
	IR + X	466	7.9	4.9030*	0.2454	421	11.9	8.1232***	2.8634
6	C	481	3.7			377	7.2		
	X	489	10.6	17.1774***		337	13.1	6.9190***	
	IR + X	555	11.5	21.5096***	1.8545	414	15.9	14.6205***	1.2554
7	C	473	6.3			382	8.6		
	X	553	12.5	11.0885***		373	13.9	5.3037*	
	IR + X	479	10.4	5.2428**	1.0727	445	12.1	2.6792	0.5982
8	C	397	7.0			298	6.7		
	X	505	10.3	2.8857		190	13.7	6.6246**	
	IR + X	466	9.2	1.3645	0.3159	336	15.5	12.1507***	0.2991
9	C	504	4.2						
	X	529	8.7	8.7444***					
	IR + X	493	6.1	1.9048	2.4802				
10	C	434	8.1						
	X	572	6.6	0.7376					
	IR + X	640	11.2	2.8994	7.7517***				

* Significant at the 0.05 level.
** Significant at the 0.02 level.
*** Significant at the 0.01 level.

treatment is significantly higher than the X-ray on the tenth day of testing in Run 1, and the X-ray is significantly higher than the combined treatment on the third day in Run 2; but there is no other occurrence of such a difference on the third day in the rest of the data and only one other suggestion of such a difference at the

TABLE 3

Crossing over in young adult females exposed to infrared radiation and gamma rays. Dose: 24 hrs. IR; 1400r

Day	Treatment	No. flies	% crov. *b-cn*	χ^2 (vs C)	χ^2 (vs γ)
1–3	C	555	2.7		
	γ	480	5.0	3.7294	
	IR + γ	323	5.6	4.7197*	0.1265
4	C	385	3.4		
	γ	252	6.0	2.3858	
	IR + γ	171	6.4	2.6465	0.0426
5	C	289	3.5		
	γ	173	6.4	2.1877	
	IR + γ	141	13.5	15.0339***	4.5046*
6	C	246	6.1		
	γ	270	19.0	18.6006***	
	IR + γ	245	11.8	5.0339**	4.8187*
7	C	370	5.4		
	γ	383	14.1	15.9324***	
	IR + γ	269	17.5	24.1775***	1.3551
8	C	382	..1		
	γ	470	16.6	17.7176***	
	IR + γ	338	14.5	10.4470***	0.6455
9	C	419	7.6		
	γ	540	13.9	9.3638***	
	IR + γ	335	13.7	7.3939***	0.0364
10	C	405	8.4		
	γ	587	10.7	1.4832	
	IR + γ	309	15.2	8.0310***	3.7967
11	C	331	7.8		
	γ	462	10.6	1.4779	
	IR + γ	279	10.8	1.5338	0.0241
12	C	349	9.2		
	γ	389	10.0	0.1600	
	IR + γ	200	9.5	0.0149	0.0418
13	C	351	10.8		
	γ	514	12.8	0.7995	
	IR + γ	268	10.4	0.0248	0.9470
14	C	354	11.6		
	γ	378	16.4	3.5036	
	IR + γ	157	9.6	0.9061	4.7659*
15–17	C	375	10.7		
	γ	550	12.0	0.3978	
	IR + γ	292	11.0	0.0155	0.2038

* Significant at the .05 level.
** Significant at the .02 level.
*** Significant at the .01 level.

tenth day (table 3). In general, when there are variations in the crossing over values between the two treated series, they tend to cancel each other in the two different runs.

Table 3 presents the data obtained with gamma ray treatment. The flies were shaken at the end of the third day and every day thereafter until the 15th day.

TABLE 4

Crossing over in young adult females exposed to infrared radiation and X-rays. Dose: 24 hrs. IR; 1400r

Day	Treatment	No. flies	% crossover.			x^2 *y-ct* region	
			y-ct	*ct-ras*	*ras-f*	(vs C)	(vs X)
1	C	192	16.7	11.5	27.1		
	X	423	17.0	9.2	25.5	0.0134	
	IR + X	166	22.9	7.8	28.9	0.0032	0.0024
2	C	499	15.8	12.0	24.0		
	X	652	16.6	10.1	26.4	0.1146	
	IR + X	667	19.8	13.2	25.2	3.0184	2.2903
3	C	574	17.1	12.7	26.1		
	X	691	21.1	10.6	25.6	3.0581	
	IR + X	739	16.4	9.6	25.6	0.1180	5.0344*
4	C	646	22.0	12.1	24.5		
	X	603	19.9	11.9	25.0	0.8162	
	IR + X	808	18.2	13.7	24.0	3.2359	0.6569
5	C	740	20.3	9.3	25.8		
	X	801	16.1	9.7	23.0	4.4886*	
	IR + X	853	15.4	10.4	25.4	6.5381**	0.1754
6	C	385	18.4	10.1	24.2		
	X	590	12.5	9.5	24.4	6.4537**	
	IR + X	485	11.1	8.9	29.1	9.3353***	0.4907
7	C	498	18.1	9.8	25.3		
	X	735	12.2	8.6	23.5	8.0868***	
	IR + X	532	13.3	8.3	20.9	4.3867*	0.3388
8	C	391	17.4	8.4	27.4		
	X	508	14.4	7.3	26.8	1.5368	
	IR + X	521	11.5	8.3	23.4	6.3668**	1.8931
9	C	495	17.4	10.5	25.9		
	X	484	12.0	8.9	22.7	5.6741**	
	IR + X	436	10.1	8.5	23.6	10.2548***	0.8182
10	C	353	16.4	7.9	24.9		
	X	568	13.6	8.8	22.0	1.4581	
	IR + X	372	12.4	10.8	22.3	2.4624	0.2855
11	C	400	19.5	11.5	21.2		
	X	548	13.7	11.3	25.7	5.7381**	
	IR + X	568	12.1	9.3	21.7	9.7827***	0.5902
12	C	398	15.1	11.1	24.6		
	X	607	13.7	9.4	21.6	0.3954	
	IR + X	438	12.1	13.2	24.9	1.5753	0.5555
All days combined	C	5571	18.2	10.6	25.1		
	X	7210	15.3	9.6	24.5	18.6160***	
	IR + X	6585	14.5	10.5	24.4	29.6036***	1.7804

* Significant at the .05 level.
** Significant at the .02 level.
*** Significant at the .01 level.

TABLE 5

Crossing over in young adult females exposed to infrared radiation and gamma rays. Dose: 24 hrs. IR; 1400r

Day	Treatment	No. flies	% Crossover			χ^2 *y-ct* region	
			y-ct	*ct-ras*	*ras-f*	(vs C)	(vs γ)
1	C	233	15.0	9.0	26.6		
	γ	104	19.2	11.5	25.0	0.9158	
	IR + γ	204	18.6	14.2	26.0	0.5589	0.0152
2	C	447	18.8	11.9	26.6		
	γ	292	17.5	13.4	26.0	0.2183	
	IR + γ	366	20.8	10.9	26.5	0.5030	1.1109
3	C	638	21.8	11.1	25.9		
	γ	558	18.3	11.1	27.1	2.3008	
	IR + γ	659	19.3	11.7	24.7	1.2716	0.1950
4	C	548	21.2	12.2	24.3		
	γ	442	19.9	9.0	23.1	0.2398	
	IR + γ	576	20.0	10.6	23.3	0.2530	0.0025
5	C	510	21.0	13.1	25.3		
	γ	337	20.5	9.2	21.7	0.0299	
	IR + γ	427	20.1	10.3	22.5	0.1052	0.0118
6	C	528	23.7	9.3	23.7		
	γ	329	20.1	11.6	21.6	1.5183	
	IR + γ	437	20.1	9.8	21.1	1.7554	0.0033
7	C	681	23.2	11.7	23.6		
	γ	557	13.4	11.7	26.2	18.9700***	
	IR + γ	412	13.1	9.0	23.5	16.7154***	0.0234
8	C	391	17.9	12.5	24.0		
	γ	426	14.3	9.9	20.9	1.9418	
	IR + γ	384	16.1	10.4	26.0	0.4237	0.5267
9	C	390	17.4	13.8	23.1		
	γ	410	15.1	9.3	24.1	0.7793	
	IR + γ	368	14.7	8.4	23.4	1.0532	0.0329
10	C	388	18.0	10.1	26.0		
	γ	410	14.4	10.7	24.1	1.9729	
	IR + γ	318	16.0	11.0	21.1	0.4915	0.3656
11	C	335	17.0	11.6	20.9		
	γ	481	16.0	12.5	24.7	0.0821	
	IR + γ	360	11.9	8.6	26.9	3.6216	2.7363
12	C	379	18.5	10.3	25.3		
	γ	330	15.2	10.3	27.9	1.4029	
	IR + γ	171	12.3	11.7	25.1	3.2755	0.7449
All days combined	C	5468	20.1	11.3	24.6		
	γ	4676	16.7	10.8	24.4	19.3529***	
	IR + γ	4682	17.4	10.4	24.0	11.8759***	0.8531

* Significant at the .05 level.
** Significant at the .02 level.
*** Significant at the .01 level.

The pattern of results is the same as that found with X-rays. Note that the increase in crossing over caused by combined treatment on the tenth day is *not* significantly different from that obtained with X-rays.

The data presented in tables 4 and 5 are those obtained with the same treatments

as above but with the first chromosome marker stock, *y cl*^{6} *ras*^{2} *f*. These experiments were undertaken to see if smaller euchromatic regions not associated with the kinetochore would show some effect of the infrared pretreatment. The data in table 4 were obtained using X-rays and those in table 5, with gamma rays. From the summary of all days tested, at the bottom of each table, it can be seen that there is little difference between the two groups. The results in table 5 show decreases in the *y-cl* region similar to those in table 4, but they are not significant in any case other than day seven. This difference in effect is due partly to the lower hatch in this experiment compared to the experiment presented in table 4. It seems unwise to conclude that there is any difference which can be attributed to the differential effects of gamma radiation on the basis of these few data.

Furthermore, it can be seen that in each case the reduction of crossing over is largely in the *y-cl* region. For these reasons, the discussion of the data will treat both tables as a unit and will be concerned with the distal region of chromosome one only. The change in crossing over follows the same general pattern shown by the *b-cn* region of chromosome two (although in opposite direction); that is, it is present, in some degree, by the fourth day and persists for an extended period thereafter. There is a slight indication of an increase in crossing over during the first day, but the numbers are small, and the increases are not significant.

DISCUSSION

There is a general tendency to consider that crossing over results from a process similar to translocation. This is the result of evidence of segmental interchange coincident with genetic exchange (STERN 1931; CREIGHTON and MCCLINTOCK 1931) Therefore, when agents which are known to increase translocation also increase crossing over, it is natural to ascribe the effect to an increase in chromosome breakage. In the work with radiations, this has been generally the case (see MULLER 1954; KAUFMANN 1954). However, there are some strong objections to attributing the modification of the rate of crossing over to increased chromosomal breakage. One of the most powerful is the *decrease* in crossing over, observed by many workers and reported in this paper, in the distal regions of the chromosome. MULLER (1954) has suggested that this decrease might either result from interference (due to increases in the proximal regions) or to irregularities of chromosome movement. These mechanisms seem unlikely, since interference of the usual type should affect the regions most strongly which are closest to the regions of increase, and since any alteration in mobility of the chromosomes should affect all regions equally. There can be little problem of synapsis, as the chromosomes are probably synapsed in the gonial stage in the Diptera.

A second objection to the breakage hypothesis as applied to radiation effects comes from the data of KAUFMANN (1941). It was shown that the breaks induced by X-radiation are distributed at random along the chromosomes of Drosophila, and that there is no tendency for the accumulation of breaks in heterochromatic regions. This would seem to contradict the breakage hypothesis, for the major effect of the radiations on crossing over induction is on regions of heterochromatin associated with the kinetochore. It may be argued that these regions are cytologically

large and that therefore any increase in breakage would show up as a pronounced effect upon genetic exchange. But this means that the radiation induced breakage is of a type that permits recombination in spite of the difficulties placed in its way by the heterochromatin, whereas "crossing over breakage" cannot. The difficulties with this argument become even greater when one considers that the heterochromatin is not completely limited to the region of the kinetochore and the major effect of the radiations is, particularly at low doses.

The above doubts are reinforced by a summary of other objections recently made by SAX (1954). "Unlike the chromosomal recombinations induced by radiation and certain chemical agents, the crossovers are always polarized and progressive from the proximal to the distal ends of the chromosome arms. Lateral fusions which give rise to dicentric bridges seldom if ever occur in normal meiosis. Yet the breakage and reunion of chromosomes induced by ionizing radiation show no evidence of polarity of the bonds between genic elements. An induced inversion is usually viable and functions as well as the original chromosome. These induced breaks, whether in sister chromatids or in different chromosomes, may also reunite laterally to give rise to dicentrics, or progressively to give rise to exchanges and translocations".

Furthermore, as the data in this paper show quite clearly, there is no enhancement of the effect of ionizing radiation when pretreated with infrared, although under these same conditions infrared will cause an increase in the number of translocations (KAUFMANN, HOLLAENDER and GAY 1946). It would appear that the infrared radiation is incapable of modifying the effects of ionizing radiation or the normal crossing over process (IVES, FENTON, YOST and LEVINE 1953). Some objection may be raised to these data in that the temperature of treatment was not quite so low as that reported by OSTER (1954). A test of this temperature effect is now under way at this laboratory. However, it must be kept in mind that the increased induction of translocations occurs with great magnitude at temperatures of 18°C or lower (KAUFMANN, HOLLAENDER and GAY 1946); therefore the major objection based on these data to the breakage hypothesis stands.

Finally, one must consider that many agents which influence crossing over (temperature, aging, inversions) are not generally considered to be agents which increase chromosome breakage. Nevertheless, in all these cases the pattern of change is the same. When one considers that in all cases of modification of crossing over the pronounced effect is always in the same regions (the area adjacent to the kinetochore or the region most distal to it), and when one considers that in most cases the effect is an increase at the kinetochore and a decrease at the distal ends, the conclusion that these various agents have a common pathway in the cell becomes inevitable. When one considers the evidence presented in this paper, coupled with the arguments from most of the work done in this field, the idea that the common pathway is chromosome breakage becomes most unlikely.

In like manner, many of the arguments presented will apply to the hypothesis that induced crossing over is the result of exchanges which occur in gonial cells (WHITTINGHILL 1954). Although the evidence is irrefutable that gonial crossing over does occur, and that in certain instances the majority of induced crossing over (even in females) can be interpreted in this way (HINTON and WHITTINGHILL 1950), the

extrapolation of the hypothesis to include all induced changes is unwarranted. Gonial exchanges could account for increases in the region of the kinetochore, but how would they account for the distal *decrease* which is compensatory to it? In some cases the effect appears too soon to be a gonial event (PLOUGH 1917). There can be no doubt that when a suppressor of normal crossing over is used (WHITTINGHILL 1954) the induced crossing over is largely gonial; however, under these conditions only that crossing over which is of a translocation nature would be expected, all other crossing over being suppressed. In view of the failure of the gonial exchange mechanism to explain the decreases observed in some regions, it would seem likely that the effect is upon the normal meiotic process, with some contribution (depending upon the agent and the situation) from gonial exchanges. Therefore, it appears that it is necessary to look for a mechanism which would permit common action by a variety of agents, and a mechanism which permits an effect of long duration. This last restriction is necessary as there can be no doubt that most of the chromosomes which exhibit the results of modified crossing over are treated in the gonial state (PLOUGH, 1924).

One mechanism which presents itself for the explanation of the induced modifications in crossing over is alteration of the coiling of the chromosomes. MATSUURA (1940) and SCHULTZ and REDFIELD (1951) have reviwed much of the evidence for this point of view. Their arguments will not be repeated here; however, some extensions may be worth while. It has been demonstrated by MATSUURA (1940) and SWANSON (1943) that the coiling of meiotic chromosomes is sensitive to both low and high temperatures. Thus relatively low amounts of energy input will produce gross changes in the structure of the chromosome. Since the chromosomes are probably in a partially coiled state at all times (the large coils of metaphase being exaggerations of a coiling at the polymer level (SAX 1954), the genetic map distance must vary with the state of the coiling pattern at all times. Since the map distances do not vary greatly from one time to another, it seems likely that the pattern of coiling (at any level) is determined with a fair degree of exactness. On this basis, we might say that the region of the kinetochore (or heterochromatin in general) (WELSHONS, 1955), is more tightly coiled than the other regions and thus exhibits a low frequency of crossing over. Any agent which could cause a relaxation of the coiling in this region would cause an increase in crossing over, and similarly if the amount of coiling is predetermined, there might be a compensatory change in the pattern distal to the kinetochore and a reduction in the number of crossover points. Thus we may make the hypothesis that agents which modify the rate of crossing over do so by an alteration of the normal pattern of coiling of the chromosome.

It is necessary to consider two things about this hypothesis. How is the degree of coiling modified, and what is meant by *coiling* in this sense? These questions can be partially answered at this time. When we speak of the chromosome, we speak of a large body composed of many molecules held together by an almost numberless set of crosslinkages. The nature of these crosslinkages is not completely clear; but there are some which are usually considered to play and which undoubtedly do play a great role in binding protein molecules together (S—S; peptide; salt linkages; etc.), others which have recently been demonstrated necessary for the joining of macro-

molecular fragments (MAZIA 1954) (bonds which involve, or are sensitive to, divalent cations), and others which probably are available to a nucleoprotein (STEFFENSEN 1955) (phosphate bridges, with or without the mediation of cations). It is known that large molecules of a polymer nature tend to fold up (taking the form of a helix, or a folded chain) as crosslinkages are formed. The greater the number of linkages the greater the degree of folding. Thus as a protein molecule coils it is bringing about more possibilities of crosslinkages, and if they are available a tightly folded molecule will result. If one permits the substitution of the words polymer, or macromolecular particle, for the word, molecule, in the preceding sentence, it is possible to consider the coiled state of the chromosome to be a reflection of the degree of "coiling" of the polymers composing it. In this loose manner one may define what is meant by coiling in the sense applied to the problem of crossing over. That is, a tightly coiled area is one which through the formation of a considerable number of linkages (or very strong linkages) has reduced the effective distance between genes, either by making pairing impossible (the genetic material being on opposite sides of the coiled strands), or by reducing the linear distance between genes.

The other question may now be answered. The effect of the agents is (directly or indirectly) upon the linkages between or within the macromolecules of the chromosome, which permit coiling to take place. A suggestion of this mechanism comes from the work on the effect of mineral changes on crossing over (BROWNING 1949; LEVINE 1955). This work indicates that agents which remove important divalent cations are able to modify the crossing over process. BROWNING (1949) observed the same compensatory behavior which is found with other agents. Therefore one is tempted to think that the effect of all the other agents is upon linkages which are cation sensitive. A recent suggestion that the chromosome contains cross linkages involving phosphate and Ca (STEFFENSEN 1955), presents an interesting possibility for the partial explanation of the observed results with induced crossing over. It is possible that the coiling of the chromosome is dependent upon cation-phosphate complexes similar to those upon which muscle action is dependent. In this case, either through direct binding of the elements by the complex or by the action of the electrostatic forces involved (MORALES, BOTTS, BLUM and HILL 1955), the degree of coiling would depend upon the amount of complexing material available and the ability of the various binding sites to compete for the material. We might then predict that removal of the material from one region of the chromosome (by any one of a number of methods) would free it to be taken up at another region, resulting in a "compensatory" change in the coiling of these two regions. The best part of such a mechanism is that it can be modified in so many different ways to produce the same general result when observed as a change in the rate of crossing over. Thus modifications can be made in the phosphate pool available to the nucleus, or in the cation concentration, or in the number, or the affinity, of the binding sites. The effects could be either directly upon the chromosome or upon the general metabolism; they might be of temporary or of long range consequence; or they might be a combination of all these things, with one factor becoming more dominant depending upon the duration of the treatment or of the amount of energy input.

It is unnecessary to carry this treatment further. With two independent variables

almost any observed result can be explained. Indeed, this hypothesis can be expanded to interpret all the observed effects of various agents upon crossing over.

It might be relevant to mention that this hypothesis permits an explanation of the interchromosomal effects observed with inversions. An inversion interferes with the normal synaptic process and causes a disarrangement of the normal coiling pattern of the inverted chromosome and its partner. This results in a change in the availability of the complexing material and thus a change in the coiling of the other chromosomes. The fact that the degree of the effect shows no direct relation to the size of the inversion (STEINBERG and FRASER 1944) is to be expected in this case, as the determining factor should be the degree of coiling mormally shown by the inverted area. Furthermore this hypothesis has the advantage that the number of chiasmata need not be limited (MATHER 1936), since a change in the complex pool will alter the total map distance so that a net increase in crossing over can occur; and it will apply to cases in which non-homologous pairing (SCHULTZ and REDFIELD 1951) can not be demonstrated.

It is important to note the following reservations. First, this is not a mechanism to explain normal crossing over. It can be expanded into such a mechanism, either by the assumption that in normal crossing over segmental interchange occurs as a result of coiling tensions within the chromosome (MATSUURA 1940) or as a result of normal dissociations in the complex-bound segments of the chromosome (MAZIA 1954). However, since a nonbreakage mechanism has some strong attractions (BELLING 1933; SCHULTZ and REDFIELD 1951) it seems wise to be cautious at this level. Secondly, it is not meant to rule out the participation of "translocation-type" exchanges in induced crossing over. It is probable that with ionizing radiations and with mineral deficiency there will be some induction of exchanges. However, we must expect many of these induced breaks to form illegitimate fusions and thus to be lost. This is particularly true of cells treated in gonial stages in which a division intervenes between treatment and division. The final product observed must be a mixture of normal crossing over, induced exchanges, and induced increases in the rate of crossing over through normal methods. For the reasons presented above, it is felt that the major contribution of modifying agents is in the last form. Thirdly, it must be borne in mind that this hypothesis is put forward to explain the results obtained in Drosophila, in which somatic synapsis plays an important role. The state of coiling will be of importance in such an organism at any stage. There is some doubt that induced crossing over would follow the same pattern in other organisms which do not show such pairing.

Finally, it must be pointed out that neither the data presented here nor those presented by IVES, *et al* (1953) are in agreement with the data of SNOAD (1955), which indicate an increase in the number of chiasmata with infrared treatment. This may be due to the difference in the organisms. Tradescantia and Drosophila do not always respond in the same way, and in the case of infrared there seems to be a temperature difference for the infrared effect (OSTER 1954). On the other hand, one might assume that in this case there is further evidence that genetic crossing over and chiasmata formation do not correspond exactly. Until studies are presented which can demonstrate an increase in chiasmata without a concomitant increase in

crossing over in one organism no further conclusions can be drawn. However, it is interesting to note that the change in chiasmata formation follows the same pattern found with changes in crossing over induced by other agents.

SUMMARY

Data are presented which show that infrared radiation delivered for 24 hours at 10°C is unable to modify the effect of ionizing radiations on the induction of crossing over. These data are considered in the light of other work on induced crossing over, and it is concluded that chromosome breakage is an unlikely mechanism to explain induced crossing over. The problem of induced crossing over is considered in the light of an hypothesis that the effect is upon the coiling pattern of the chromosomes. It is suggested that the use of infrared radiation may be one way to determine the correspondence between chiasmata formation and genetic crossing over.

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RADIATION-INDUCED PRESUMED SOMATIC MUTATIONS IN THE HOUSE MOUSE[1]

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EXPERIMENTS on the induction of somatic mutations can provide information in at least two main fields of interest: (1) in the comparative study of mutation induction in different types of cells; and (2) in the study of cell lineage, where the induced somatic mutations can be used as a valuable tool.

Somatic mutations involving specific loci have been repeatedly produced by radiation in Drosophila (PATTERSON 1928, 1929a, 1929b, 1930; TIMOFÉEFF-RESSOVSKY 1929; GULBEKIAN 1934; HASKINS and ENZMANN 1937; LEFEVRE 1948, 1950) and in plants (e.g., SPARROW *et al.* 1952). In mammals, however, although several dozen spontaneous coat color mosaics have been observed (CASTLE 1922; WRIGHT and EATON 1926; BITTNER 1932; DUNN 1934, review; FELDMAN 1935; BHAT 1949; CARTER 1952; MORGAN and HOLMAN 1955), the induction of mosaics by irradiation has not been reported except in our preliminary communication (RUSSELL and MAJOR 1952). Consequently, several points of technique had to be worked out prior to the start of the experiment. The method adopted makes it possible to distinguish the genetic effects produced from developmental effects of radiation which might otherwise obscure them.

The results indicate that somatic mutations at specific loci were induced by radiation in prospective pigment cells. The mutation rate in these cells could be calculated and compared with the rate found in mouse spermatogonia for the same loci (W. L. RUSSELL, 1951). Various features of the results suggest some working hypotheses and some tentative conclusions about cell lineage in mouse development.

MATERIALS AND METHOD

In summary, the method consisted of irradiation of embryos heterozygous for four coat color genes and examination of the adult fur for mosaic patches. A parallel control series involved irradiation of embryos homozygous for the wild type alleles of the four loci studied. Spots of altered color in the latter series should represent mutations to dominants anywhere in the genome, or else non-mutational, i.e., developmental, changes (e.g., abnormal differentiation of pigment cells). When the frequency of these is subtracted from the total mosaics in the heterozygous series, one should be left only with changes due to the expression of the four coat color recessives.

Stocks and mating system

Females of the inbred C57BL strain $\left(\frac{a}{a}\ \frac{+^{p}+^{c}}{+^{p}+^{c}}\ \frac{+^{d}+^{se}}{+^{d}+^{se}}\ \frac{+^{b}}{+^{b}}\right)$ were mated either to inbred NB strain males $\left(\frac{a}{a}\ \frac{p\ c^{ch}}{p\ c^{ch}}\ \frac{d\ se}{d\ se}\ \frac{b}{b}\right)$ or to C57BL males. (a = non-agouti;

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p = pink-eye, diluted coat color; c^{ch} = chinchilla; d = dilution; se = short ear, no effect on coat color; b = brown). Offspring of both types of mating are black. The first litters raised served as non-irradiated controls. Following weaning of the first litters each female was returned to her original mate and the second copulation was timed by observation for vaginal plugs. Irradiation was administered during the second pregnancy. From 65 irradiated females, 60 litters were obtained. The number of unirradiated litters (i.e., first litters) observed was 57.

Stage irradiated

In a somatic mutation experiment, each individual observed represents a large number of cells in which a mutation could have occurred, instead of a single cell as in a germinal mutation experiment. It is, therefore, of advantage to irradiate embryos at a time when the number of pigment precursor cells is already large. However, if this number is *too* large, the spot resulting in the adult fur will be too small to be perceptible in every case.

To determine the embryonic stage that would best meet these criteria, a preliminary experiment was carried out. C57BL females, mated to NB males, were irradiated in various stages of pregnancy, ranging from day $6\frac{1}{2}$ to day $12\frac{1}{2}$ postfertilization, and 136 young were raised. Mosaic spots in the coat were observed in four animals, all of which had been irradiated as day-$10\frac{1}{2}$ embryos. (One spot was observed among 75 control young.) It was concluded that mosaics induced in embryos older than $10\frac{1}{2}$ days were too small to be perceptible, while the frequency of mosaics produced by irradiation of younger embryos was not high enough to have produced a case in the preliminary experiment and, thus, not high enough to be practical. It was, therefore, decided to irradiate embryos at $10\frac{1}{4}$ days, i.e., on the morning of the 10th day following observation of the vaginal plug.

Dose

The stage determined to be most suitable from the point of view of mosaic production is, unfortunately, also the most sensitive to radiation with respect to incidence of neonatal death (RUSSELL and RUSSELL 1954; RUSSELL 1954). Since 200r at $10\frac{1}{2}$ days postfertilization was already known to cause about 70 per cent neonatal death (probably no prenatal death), the present experiment was begun with 150r. With that dose, the survival of animals to an age at which they could be observed for coat color mosaics was found to be slightly lower than two thirds of the control value (table 1). With two thirds of that dose, i.e., with 100r, survival was almost equal to controls. Thus, from the practical point of view of yield per effort, the two doses should be equivalent. However, since it seemed probable that complications from factors such as abnormal differentiation would be greater with 150r, the bulk of the data was obtained with 100r.

Radiation factors were as follows: 250 kvp X-rays, delivered by a Coolidge self-rectifying tube, half-value layer of 0.4 mm copper, inherent filtration 3 mm aluminum. Using a current of 15 milliamperes, the intensity obtained at 84 cm distance was 91 ± 3r per minute. The pregnant females were exposed in individual compartments of a perforated lucite box resting on a 10.8-cm-high masonite scatter block.

TABLE 1

Survival of mice irradiated 10¼ days postconception

	C57BL ♀ × NB ♂			C57BL ♀ × C57BL ♂		
	0r	100r	150r	0r	100r	150r
Number of ♀ ♀ treated	31	38[b]	4	25	16	7[c]
Number of young born	211	255	34	153	89	35
Young born/litter	6.8	7.5	8.5	6.1	5.6	5.8
Percent survival to observation[a]	95.7	92.2	61.8	92.2	92.1	57.1

[a] Detailed observation for coat color mosaics was made between 32 and 58 days of age (see text).

[b] Includes four females that had no litters, probably because of misdiagnosis of pregnancy.

[c] Includes one female that had no litter.

Observation

Early in the experiment, the first observation for coat color mosaics was at 1–2 weeks of age, but while four of the mosaics were noted at that age, eight others, visible at a later age, were missed, probably owing to the hair, particularly in ventral regions, not yet being full length. Detailed examination of the animals was, therefore, made sometime between 32 and 58 days of age. Non-mosaic animals were discarded at that time. Mosaic animals were saved and again observed 4–10 weeks later to insure that the spots were not transient, e.g., due to moulting patterns. An additional observation was made on a few mosaic animals at about one year of age but it was found that old-age graying occasionally obscures the spots at that age.

The following records were made, at the time of the first or second observation of the mosaic: sketching of the position of the spot(s); outline of the spot(s); estimate of the proportion of spot area occupied by mutant hair; and gross description of the spot color. Hairs were plucked for histological examination. Measurement of the spot with calipers and tracing of the outline on squared paper to determine mosaic area were always done in fully mature mice, namely, between 8 and 21 weeks of age.

The area of the total coat was measured in a few skins and found to be about 45 cm^2. Since small variations in total coat area will not markedly affect the calculated proportion of coat occupied by the spot, the total coat area was not determined for each individual mosaic mouse but taken to be 45 cm^2 in all cases.

Small white belly spots, which occurred in the C57BL × C57BL matings, were recorded only if the spot was made up of at least five hairs.

The ears of all animals were examined, since offspring of the C57BL × NB cross are heterozygous for short-ear, *se*. A few of the animals were examined at birth for possible changes in the eyes. Somatic mutations involving eye tissue should be observable for three of the loci: *p* (*p*/*p* = pink eyes), *c*, and *b* (c^{ch}/c^{a} and *b*/*b* both reduce the width of the pigment ring at birth).

RESULTS

1. Frequency of mosaics

Among 701 animals whose adult coats were examined, there were 31 with altered spots of coat color. This excludes small white mid-ventral spots which will be dis-

TABLE 2

Frequency of various spots in the coats of mice that had been irradiated at 10¼ days postconception

	C57BL ♀ × NB ♂			C57BL ♀ × C57BL ♂		
	0r	100r	150r	0r	100r	150r
Number of animals observed:						
♂♂	104	110	9	70	50	9
♀♀	98	125	12	71	32	11
Total	202	235	21	141	82	20
Percent with spots of altered coat color:[a]						
♂♂	1.0	14.5	(0)	0	2.0	(11.1)
♀♀	0.0	8.0	(8.3)	1.4	0.0	(0.0)
Total	0.5	11.1	(4.8)	0.7	1.2	(5.0)
Percent with white midventral spots:						
♂♂	0.0	0.0	0.0	2.9	22.0	33.3
♀♀	0.0	0.0	0.0	9.9	34.4	63.6
Total	0.0	0.0	0.0	6.4	26.8	50.0

[a] Excluding white midventral spots listed below.

cussed below (sec. 5). The frequency of mosaic animals in the various groups is shown in table 2. The five mosaics found in the preliminary experiment (see Methods) are not included in this table.

The 150r groups are small (see Methods) and will be discussed no further from the point of view of frequencies. In offspring of the C57BL × NB cross that had been irradiated with 100r as 10¼-day embryos, the incidence of mosaic animals was about 11 percent. This incidence is significantly higher than the 0.5 percent found in the controls of the same cross ($t = 4.6$; $P \ll 0.01$) and also significantly higher than the 1.2 percent found in offspring of C57BL × C57BL matings irradiated with 100r ($t = 2.8$; $P < 0.01$). The difference between the sexes in the C57BL × NB, 100r data is not significant ($t = 1.6$; $0.1 < P < 0.2$).

If p = spontaneous frequency of animals with coat color changes due to developmental causes or to somatically arisen coat color dominants,

q = 100r-induced frequency of animals with coat color changes due to developmental causes or to somatically arisen coat color dominants (it is assumed that q is identical in C57BL × C57BL homozygotes and C57BL × NB heterozygotes),

r = spontaneous frequency of animals with somatic expression of the recessives at the *b*, *c*, *p*, and *d*-loci,

s = 100r-induced frequency of animals with somatic expression of the recessives at the *b*, *c*, *p*, and *d*-loci,

then, p = frequency of mosaic animals in the (C57BL × C57BL), 0r group . . . (1)

p + q = frequency of mosaic animals in the (C57BL × C57BL), 100r group . . (2)

p + r = frequency of mosaic animals in the (C57BL × NB), 0r group . . . . . (3)

p+q+r+s = frequency of mosaic animals in the (C57BL × NB), 100r group. (4).

Therefore, the frequency of animals with somatic expression of the recessives at the *b*, *c*, *p*, and *d*-loci induced by 100r can be obtained from (4)−(3)−[(2)−(1)], i.e., 0.1106 − 0.0050 − (0.0122 − 0.0071), or 0.1006 ± 0.0253. It should, of course, be remembered that this frequency applies to animals irradiated as $10\frac{1}{4}$-day old embryos, i.e., having spots averaging a definite proportion of the coat (see below): earlier or later irradiation would, within limits, be expected to give lower and higher frequencies of mosaic animals, respectively.

To determine whether or not the mosaic animals are distributed randomly among litters, it is necessary to test several littersizes simultaneously since, for any one littersize in the C57BL × NB, 100r group, there are never more than nine litters. Two independent statistical methods were used[2], each of which simultaneously tested littersizes of 6, 7, 8, and 9 (a total of 26 litters, or 203 animals). By neither of the methods did the mosaics show a significant departure from random distribution among litters.

2. Appearance of mosaic spots

The outline of mosaic spots was, in most cases, extremely diffuse and could not be described by simple geometrical terms, such as circular, triangular, etc. Often the mutant hairs were concentrated into two or even more main areas which, however, in all but two animals were either adjacent or were close to each other with a few mutant hairs bridging the gap. In two animals there was a complete gap between main areas of mutant hair, but the areas were of the same color and the gap no larger than one cm. It may, therefore, be assumed that each mosaic animal observed in this study represents but a single event of change.

Within mutant areas, there were varying degrees of mixture of mutant and apparently nonmutant (i.e., black) hairs. This is illustrated in figure 1. Whether there is a mixture of mutant and nonmutant cells within individual hairs has not yet been determined.

The following colors of mutant spots were recorded from gross observation: dark gray (two animals); gray (seven animals); light gray or gray-white (nine animals); brown or tan (three animals); yellow-brown (nine animals). It is obviously very difficult to give accurate color descriptions for small spots which, moreover, consist of mixtures of various proportions of mutant and nonmutant hairs. Considering only known alleles at the loci studied, and their action on an a/a background, spots representing mutations at the *b*-locus should appear brown; at the *c*-locus from dark gray to quite light gray; at the *d*-locus blue-gray; and at the *p*-locus medium gray. From gross examination of spots alone, it therefore seems probable that the colors recorded represent mutations at at least two of the loci, namely, *b* and *c*. From histological examination of hairs, which is difficult (because of hair mixture) and not yet completed, it can be stated that the *p*-locus was definitely involved in at least one case. This case, grossly classified as "gray-white" was, furthermore, interesting in that the pigment in the mutant hair appeared as in $\frac{+\ p}{c^{ch}p}$, rather than as in $\frac{c^{a}p}{c^{ch}p}$, as it presumably would have done in case of somatic chromosome loss or deletion large

[2] We are grateful to Dr. A. W. Kimball of the ORNL Mathematics Panel for carrying out these tests.

FIGURE 1.—An example of one of the larger mosaics. This animal, ♂636, was an offspring of the C57BL × NB cross and was irradiated with 100r X-rays, 10¼ days postconception. The mutant spot occupied 0.0093 of the body surface and was grossly classified as "light gray."

enough to involve both *c* and *p*-loci (crossover distance between *c* and *p*: 16 in females, 12 in males).

3. Location of mosaic spots

For the most part, spots appeared to be randomly distributed over the mouse surface, as shown in figure 2, which is a composite drawing showing the location of all nonblack areas (other than white mid-ventral spots) in offspring of the C57BL × NB cross. However, it was also clear that some spots represented abnormal differentiation, rather than genetic change. Thus of the three spots observed in offspring of C57BL × C57BL matings, two had some connection with nipples: one (in the 0r group) was an extension of the yellow-brown group of hairs which normally surrounds the female nipple; the other (in the 100r group) was the development of such a nipple spot in a male. In the C57BL × NB cross, also, there was one case of nipple-spot extension in a female. In addition, there were two other cases of yellow-brown spots close to normally yellow-brown areas: one on the snout; and one anterior to the penis. These three cases are, however, included with the rest for all quantitative purposes, since ideas as to their origin are only speculative and the C57BL × C57BL matings presumably provide an adequate control for color changes due to abnormal differentiation rather than expression of the recessives.

No mosaic eyes were observed. However, while pink-eye mosaic eyes might be detectable, it is exceedingly doubtful that small sections of b/b or c^{ch}/c^{a} tissue would

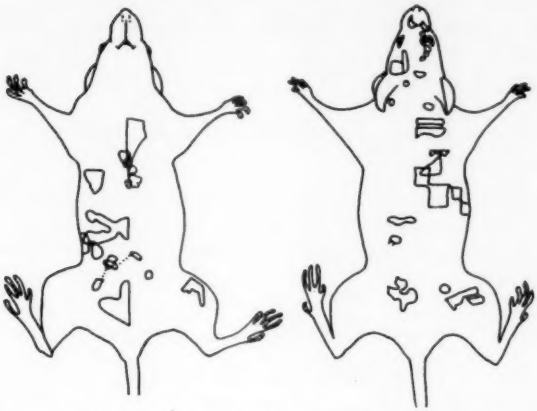

FIGURE 2.—Composite drawing showing location of spots in all mosaic offspring of the C57BL × NB cross.

show up in the adult black eye; and the number of animals observed young enough to reveal possible changes in the pigment ring was not very large.

All ears examined were normal. There was thus no evidence for somatic expression of the heterozygous *se*.

4. Proportion of coat occupied by mosaic spot. Mutation rate

A certain degree of inaccuracy entered into determination of spot size, owing to the fact that the spot perimeter was diffuse and that, moreover, the percentage of mutant hair within this indistinct perimeter could only be estimated. The proportion of adult coat occupied by mutant hair, which was computed for each animal, is therefore only approximate. The reciprocal of this proportion may be taken to give an estimate of the number of prospective pigment cells that were present at the time the mutation, or other change, occurred. Such an estimate will be valid only if the cell in which the ultimately visible change was induced does not contribute differentially less progeny on account of that change. However, such an assumption can probably be made with relative safety (see Discussion).

Table 3 shows the distribution of mosaic animals in the various groups with respect to proportion of adult coat occupied by hair of altered color. The total range for 30 mosaic animals was 0.000173 to 0.0145. If the reciprocals of these figures are taken to represent prospective pigment cells present in day-$10\frac{1}{4}$ embryos, the extremes of the range, 69–5769, are separated by approximately six cell divisions.

The probability of finding a mosaic in a given group does not depend solely on number of embryos in that group and on mutation rate, but also on number of prospective hair pigment cells per embryo. Therefore, the distribution of mosaics shown in table 3 does not directly indicate the distribution of day-$10\frac{1}{4}$ embryos with respect to number of prospective pigment cells. This latter distribution, which is

plotted in figure 3, may be derived as follows, using the results of the C57BL × NB, 100r group:

Let $p_1, p_2, \ldots$ etc. = proportion of total fur having altered color (in individual mosaic animals);

$n_1, n_2, \ldots$ etc. = number of mosaic animals characterized by $p_1, p_2, \ldots$ etc., respectively;

$\frac{1}{p_1}, \frac{1}{p_2}, \ldots$ etc. = number of prospective pigment cells present at the time the ultimately visible change occurred;

x = rate of ultimately visible change/cell/100r

$$= \frac{\text{altered cells}}{\text{total cells}}.$$

Since each mosaic animal was probably due to a single altered cell, then, for embryos of any given number, $\frac{1}{p}$, of prospective pigment cells,

$$x = \frac{n}{\frac{1}{p} \times \text{number of embryos with } \frac{1}{p} \text{ cells}}$$

(it is assumed that x is the same for all values of $\frac{1}{p}$ encountered in this experiment), and $\frac{p_1 n_1}{x}, \frac{p_2 n_2}{x}, \ldots$ etc. = number of embryos having $\frac{1}{p_1}, \frac{1}{p_2}, \ldots$ etc., prospective pigment cells. x was calculated from the results of the C57BL × NB, 100r group, in which 235 animals were observed:

$$x = \frac{1}{235}(n_1 p_1 + n_2 p_2 + \cdots)$$

$$= 3.1 \times 10^{-4}$$

In this calculation, the $p_1, p_2, \ldots$ etc. values used were for individual animals rather than for the means of the classes shown in table 3. (It may be noted that if a few animals with very small spots, i.e., p = very small, were missed in observations, this would not appreciably affect x, and thus not affect the mutation rate, calculated from x below.)

Figure 3, based on the above calculations, plots the distribution of embryos with respect to number of prospective pigment cells. The geometric means of the ranges in the left-hand column of table 3 were used for the abscissa $\left(\frac{1}{p}\right)$. The ordinates are thus $\frac{np}{x}$, where n denotes the frequencies shown in table 3. It may be noted that the curve in figure 3 indicates the modal number of prospective pigment cells in day-10¼ embryos to be between 150 and 200. The left-hand portion of the curve, however, cannot be drawn from the data since the number of embryos irradiated was not large enough to give mosaics in the groups that have such low cell numbers that the chance of producing a mosaic animal is very small. If the distribution is symmetrical then the total number of embryos with low cell numbers is not very large.

TABLE 3

Distribution of mosaic animals with respect to proportion of adult coat occupied by hair of altered color[a]

Area of altered color / total area (= 45 cm²)	C57BL ♀ × NB ♂			C57BL ♀ × C57BL ♂		
	0r	100r	150r	0r[b]	100r	150r
0.000160–0.000319	—	4	—		—	—
0.000320–0.000639	—	4	—		—	—
0.000640–0.001279	1	2	—		1	1
0.001280–0.002559	—	7	—		—	—
0.002560–0.005119	—	5	—		—	—
0.005120–0.010239	—	3	—		—	—
0.010240–0.020479	—	1	1		—	—

[a] White midventral spots (see table 2) are not included in this table.

[b] One mosaic animal occurred in this group but is omitted from the table since its spot was not accurately measured.

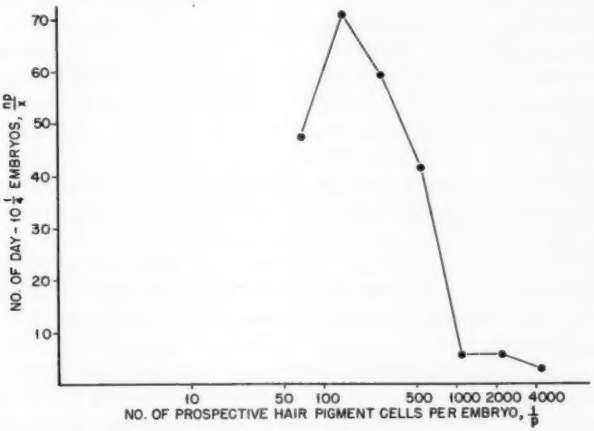

FIGURE 3.—Calculated distribution of day-10¼ embryos with respect to number of prospective hair pigment cells (see Results, sec. 4).

The mutation rate—or rate of any other change leading to expression of the four coat color recessives for which the C57BL × NB animals are heterozygous (see Discussion)—may be calculated as follows. The rate of ultimately visible change/cell/100r was shown to be 3.1×10^{-4}. It was calculated in section 1 of the Results that the frequency of mosaic animals due to radiation-induced expression of the recessives at the four loci is 0.1006, while the frequency of all animals with spots in the C57BL × NB, 100r group is 0.1106. Thus, the mutation rate/locus/r $= 3.1 \times 10^{-4} \times \frac{0.1006}{0.1106} \times \frac{1}{4} \times \frac{1}{100} = 7.0 \times 10^{-7}$. The 95 percent confidence interval, assuming no error in x, is $(4.66, 9.18) \times 10^{-7}$. Assuming 50 percent error in x (due to difficulty of spot size determination), the 95 percent confidence interval is $(2.51, 11.33) \times 10^{-7}$.[2]

5. White midventral spots

As shown in table 2, small white midventral spots occurred only in offspring of C57BL × C57BL matings. The increase from 6 percent in the controls to 27 percent in the group that had received 100r at $10\frac{1}{4}$ days of development is clearly significant ($t = 4.3$; $P \ll 0.01$); and the further increase from 27 percent to 50 percent between the 100 and 150r groups is significant at the $P = 0.05$ level ($t = 2.0$). It is interesting to note that in all three groups—0r, 100r, and 150r—the proportion of white midventral spots is higher in females than in males.

White midventral spots could not be produced by irradiating embryos of the C57BL × NB cross.

DISCUSSION

It seems clear from the comparison of the heterozygous (C57BL × NB) and homozygous (C57BL) groups (see Results, sec. 1) that at least a large proportion of the mosaics were due to expression of the four coat color recessives (*b*, *c*, *p*, and *d*) for which the C57BL × NB animals are heterozygous. Whether, however, this expression is due to somatic mutation of the wild-type allele or to other causes cannot be definitely stated on the basis of the present results. In the case of the spot which was diagnosed as phenotypically $p+^{c}/pc^{ch}$, on the basis of histological examination of hair pigment (♀ 595), some of the alternatives to somatic mutation may be ruled out. Since *p* and *c* are linked (crossover distance: 16 in females, 12 in males), this spot could not have been due to loss of a chromosome, or to somatic reduction (which would give rise to haploid cells in the spot). It could be due to somatic nondisjunction *only* if the trisomic $pc^{ch}/pc^{ch}/+^{p}+^{c}$ produces a $p+^{c}/pc^{ch}$ phenotype. It could be due to position effect of a break near the *p*-locus, but position effects have never been demonstrated in a mammal. It could be due to somatic crossing over (CARTER 1952, has suggested that spontaneous somatic crossing over can occur in mice), but only if *p* is distal to *c* and the crossover occurred between them, if *p* is proximal to *c* and there was a double crossover, or if *p* and *c* are on opposite sides of the centromere with a crossover occurring between them. Thus, while a few mechanisms by which the recessives come to express themselves can be ruled out on the basis of mosaic ♀ 595, more than one possibility remains. In the absence of further evidence, it would seem most conservative to think of the mosaics in this experiment as being due to somatic gene mutations or small deficiencies involving the loci under study.

If, in fact, the mosaics observed in the present experiment are due to somatic mutations, the somatic mutation rate (see Results, sec. 4) is 7.0×10^{-7}/r/locus [95 percent confidence limits $(2.51, 11.33) \times 10^{-7}$, assuming 50 percent error in measurement of spot size]. For comparison, the germinal rate, in spermatogonia, for the same loci (*b*, *c*, *p*, and *d*) is 2.4×10^{-7}/r/locus (W. L. RUSSELL 1951); or 2.1×10^{-7}/r/locus if one omits mutations to intermediate alleles, which are probably not included in the somatic rate (a small spot of only slightly altered color would certainly have been missed). Even though the somatic rate is open to some doubt due to the difficulty of obtaining an exact measure of the proportion of mouse surface occupied by mutant area, it is probably safe to say of the comparison that, for the

same four loci, the germinal rate induced in spermatogonia and the somatic rate, induced in $10\frac{1}{4}$-day embryos, are of the same order of magnitude. LEFEVRE (1950) found similarity between induced somatic and germinal rates at the *w*-locus in *D. melanogaster* and took this as evidence that X-ray-induced mutations differed qualitatively from spontaneous ones, since, as he says, "the spontaneous mutation rate of a given gene . . . is frequently sensitive to the influence of its cellular environment." Although the rough similarity of the X-ray-induced somatic rate to the spermatogonia rate, found in the present experiment, resembles LEFEVRE's result, his conclusion seems open to some doubt in view of evidence from other sources that there may be variation in radiation-induced rate depending on cell type irradiated. Thus, it has recently been shown that the rate of X-ray-induced changes affecting specific loci is different at different stages in gametogenesis (ALEXANDER 1954; W. L. RUSSELL 1956 and unpublished).

In calculations of the somatic rate, the size of the cell population that was irradiated is not observed directly. One assumption that must be made is that the cell in which the scorable change was induced does not contribute differentially less progeny on account of that change; for, if it does, the determined rate will be a minimum. It seems, however, unlikely that this is the case, since it may be calculated (making reasonable assumptions with regard to number of loci) that most other somatic cells would have at least one mutation somewhere in the genome induced by 100r and would, therefore, on the average, be equally affected.

It is also necessary to consider inequality of cell contribution to the ultimately observed structure (here hair) that is due to normal cell lineage. Of all the cells of a $10\frac{1}{4}$-day embryo that will provide some descendant cells contributing to the adult hairs, not all necessarily make equal contributions. Thus, e.g., cell A may contribute its entire progeny, while cell B may contribute only a very small part, the rest of its progeny producing some other tissues. In the use of the mutant coat/total coat ratio to compute the size of the cell population at the time of irradiation, such a situation would lead to error in *individual* cases, but the errors should cancel out over several cases if the chance of causing mutation in cells A and B was equal.

The material used in this experiment was not suitable for determining whether the mosaics involved germinal as well as somatic tissue. Since the embryos were already heterozygous for the genes in question, gonadic mosaics would not have produced new types, except in the case of (a) mutations to new, phenotypically distinguishable, alleles, and (b) changes at the *d*-locus that did not involve the closely linked $+^{se}$. In all other cases, probably the great majority, the mosaic portion of the gonad would simply carry one of the genes in homozygous instead of heterozygous condition. Since it seems very unlikely, from the evidence discussed below, that the mosaic portion of the gonad could be large, the contribution from this portion would be insufficient to upset the normally expected 1:1 ratio detectably, except with very numerous progeny from each individual mosaic. Moreover, unless one knew which of the loci was involved, one would have to test simultaneously for all of them, with a resulting 16 genotypes, some of which could be established only by further testing. In another investigation, involving spontaneous somatic reverse mutations at the *pe*-locus (RUSSELL and MAJOR 1956), i.e., a situation in which each individual

descendant from mutant portions of the gonad can be positively identified, none of 19 animals with coat color spots of approximately the size induced in the present experiment was found to have gonadal tissue involved. It may, therefore, be suggested that the radiation-induced somatic mutations in the present experiment were induced too late for the cell lineage to contribute to gonadal tissue. This also seems probable on embryological grounds.

It appears from the work of RAWLES (1947) that, at the stage at which radiation was administered in the present experiment, the cells which will ultimately become melanophores have already become determined. By day $10\frac{1}{4}$, prospective pigment cells (probably neural crest cells) have begun to spread laterally from the dorsal midline and may have reached the somite region, at least in the anterior parts of the embryo. It appears from calculations shown in figure 3 that the modal number of prospective pigment cells present at the time of irradiation (day $10\frac{1}{4}$) is between 150 and 200. These cells probably have a rather rapid division rate, since the extremes of the range of spot sizes observed could be accounted for by cell numbers differing by six divisions. Since embryos of the same chronological age may differ as much as 24 hours in developmental age (ALLEN and MACDOWELL 1940), one must conclude that, at that stage, mitoses in prospective hair pigment cells are separated by only 4-hour intervals (or even less if there were embryos with cell numbers outside the observable range). In spite of this rapid division rate, there is apparently a limit to cell mixing, since, even in the case of mutations that occurred early (i.e., animals with a high proportion of mutant hairs), there was always one main mutant area, albeit a diffuse one. The present results indicate that, in general, dorsal spots tend to be somewhat more diffuse than ventral ones, but the difference is not large and more data are needed.

Wright has pointed out that in tortoise-shell guinea pigs the boundaries between red and black areas are sharp if the spotting factor, *s*, is present. In spontaneous rodent mosaics reported in the literature, the outlines of the mutant spot also appear sharper when animals have white spotting due to the genes *s* or *bt*; and DUNN (1934) has pointed out that the mosaic spots often occur in the white-spotting areas. An experiment is underway to compare radiation-induced mosaics on spotted and self backgrounds.

On the C57BL × NB background, no white spots were produced (although the occurrence of occasional white hairs in the light gray spots cannot be ruled out). It would, therefore, appear either that irradiation on day $10\frac{1}{4}$ causes no killing of prospective pigment cells; or, that if some of these cells *are* killed, their places can be filled in by a surplus of surviving cells. The latter hypothesis is supported by the results of the C57BL × C57BL matings. This genetic background (in contrast to C57BL × NB) may be thought of as normally providing only barely enough pigment cells to fill up the surface, since in about six percent of the animals the last region in which the pigment cells meet in their latero-ventrad migration is left deficient, and a small midventral white spot results. Since 100r to embryos of the C57BL × C57BL matings increases the incidence of these midventral white spots to 27 percent, some pigment cell killing must be assumed; and since the radiation causes no white spotting elsewhere on the body, it may be suggested (if RAWLES' time relations can be applied

to our case) that there is some mechanism that insures that no gaps are left during prospective pigment cell migration from the neural crest; and that, as a result of this, the deficiency is only noted at the end of migration, i.e., midventrally.

SUMMARY

1. Embryos of a C57BL × NB cross, heterozygous for four coat color genes, were irradiated with 100 or 150r of X-rays at $10\frac{1}{4}$ days postconception, and the adult fur was observed for mosaic patches. A parallel control series involved irradiation of embryos of C57BL × C57BL matings, homozygous for the wild-type alleles at the four loci. Non-irradiated offspring of both types of mating were also studied. Altogether, 701 animals were observed.

2. Two types of spots were found on the normally black coats: (a) nonwhite spots, distributed randomly over the mouse surface, which occurred, with different frequencies, in all groups studied (see below, points 3–7); (b) small white spots which were always midventral and which were found only in C57BL × C57BL matings. While the latter type of spot occurs rather frequently spontaneously, its incidence is significantly increased by irradiation. This is interpreted as indicating that radiation causes some killing of prospective pigment cells but that this becomes apparent only in a genetic background (such as our subline of C57BL) that normally provides only a barely sufficient number of such cells.

Subsequent points of the summary apply to nonwhite spots only.

3. Nonwhite spots were, for the most part, distributed randomly over the mouse surface. However, it was also clear that some spots, e.g., those adjacent to nipples, represented abnormal differentiation, rather than genetic change. The three types of controls used probably make proper allowance for such spots.

4. The frequency of mosaic animals in the C57BL × NB, 100r group was 11.1 percent. After taking account of the three types of controls, the frequency due to expression of the recessive at one or another of the four loci was calculated to be 10.1 percent for 100r.

5. The mutant areas were, in most cases, rather diffuse and were occupied by varying degrees of mixture of mutant and probably nonmutant hairs. The colors observed are consistent with the view that the various mosaics represent the expression of one or another of at least three, and probably all four, of the loci studied.

6. The proportion of the coat occupied by mutant hair ranged from 0.0145 to 0.000173 in different mosaic animals. Assuming that the reciprocals of these figures may be taken to represent prospective pigment cells present at the time of irradiation, the extremes of the range are separated by six cell divisions. It can be calculated that the modal number of prospective pigment cells in $10\frac{1}{4}$-day embryos is between 150 and 200.

7. It is clear from the results that most of the mosaics were due to expression of the coat color recessives for which the C57BL × NB animals are heterozygous. At present, one cannot be certain as to the mechanism by which these recessives come to express themselves. Several mechanisms are ruled out, but more than one possibility remains. In the absence of further evidence, it seems most conservative to consider the mosaics as due to somatic mutations or to small deficiencies involving

the loci under study. The somatic mutation rate can be calculated as 7.0×10^{-7}/r/locus, and 95 percent confidence limits, assuming 50 percent error in measurement of spot size, are $(2.51, 11.33) \times 10^{-7}$. The germinal rate induced in spermatogonia (W. L. RUSSELL 1951) for the same four loci is 2.4×10^{-7}/r/locus.

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THE INDUCTION OF CHROMOSOME ABERRATIONS IN TRADESCANTIA BY ULTRAVIOLET RADIATION[1]

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DIRECT cytological studies of ultraviolet-induced chromosomal aberrations, in contrast to the mutagenic effects of this agent, have been few in number and limited in extent. The meager literature on the subject has been summarized by SWANSON and STADLER (1955), and it deals primarily with aberrations in Tradescantia pollen-tube chromosomes. In most other organisms possessing chromosomes suitable for cytological examination, induction of nuclear damage by ultraviolet radiation is extremely difficult owing to the strong absorption and scattering in this region by overlying tissues or cellular constituents. Notable exceptions to this state of affairs are the studies of STRAUB (1941), SLIZYNSKI (1942), and the recent paper of FABERGÉ (1956). The pioneer work on Tradescantia was done by SWANSON (1940, 1942, 1943, 1944), who observed simple chromatid deletions but no isochromatid or exchange aberrations after irradiation of growing pollen-tube cultures. Most of these investigations were carried out with monochromatic radiation of wave-length 2537 Å; in a few instances, mixed radiation in the near ultraviolet was used. After these studies by SWANSON, the subject has been largely ignored experimentally until the work by LOVELACE (1954), a surprising neglect in view of the cytological suitability of Tradescantia chromosomes, the accessibility of the pollen or pollen tube nuclei to ultraviolet radiation, and the development of reliable and routine pollen culture techniques (BISHOP 1949; CONGER 1953). LOVELACE (1954) treated Tradescantia dry pollen with 2650 Å ultraviolet radiation and succeeded in producing simple chromatid breaks, isochromatid breaks, and chromatid/chromatid interchanges, i.e., the customary types of chromatid breaks induced by ionizing radiation, as well as complete fragmentation of entire chromatid arms and, often, entire chromosomes. The contrast between the findings of SWANSON on the one hand and those of LOVELACE and the present authors on the other are not too surprising in view of the fact that the ultraviolet treatments were given under quite different conditions, i.e., growing pollen tubes versus dry pollen.

The investigations cited were primarily qualitative and were concerned mainly with the question of whether ultraviolet radiation is able to induce chromosome breakage. After LOVELACE'S observation (1954) that ultraviolet treatment of dry pollen does result in chromosomal aberrations in the pollen tube division in Tradescantia, a further investigation of this phenomenon, including the study of factors governing ultraviolet-induced chromosomal damage and a comparison of the results of ultraviolet treatment with those produced by ionizing radiation, has appeared highly desirable. Brief preliminary accounts of some aspects of these studies have been reported in abstract form (KIRBY-SMITH 1954; KIRBY-SMITH and CRAIG 1955).

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The present paper represents the complete account of our work in these problems. It also includes a semiquantitative determination of the action spectrum for chromosomal breakage by ultraviolet radiation. Among the requirements for a reliable wave-length experiment of this type, as well as for other more quantitative studies, are: (1) a knowledge of the time-intensity or reciprocity relations; (2) definite proof that breakage by ozone is not a contributing factor to the results; and (3) a determination of the role of any possible photorecovery processes. A study of these possible modifying factors has been made in addition to work on the larger problem of the effects of wave length and dose.

EXPERIMENTAL METHODS

Dry pollen from *Tradescantia paludosa* Anderson and Woodson, Sax clone 3, was used in all experiments. The handling of the pollen was facilitated and clumping was prevented by desiccating the pollen over anhydrous $CaSO_4$ for approximately thirty minutes immediately after collection. After drying, the pollen was dusted in a monolayer over the surface of a glass slide 0.5 x 2.0 cm. At this point, the preparations were examined under a low-power dissecting microscope and any clumps of pollen were removed with a needle, thus ensuring that no pollen grains were shielded from the incident radiation. The slides were then placed in the irradiation chamber shown schematically in figure 1, and the pollen was subjected to ultraviolet treatment.

All irradiations were carried out at constant temperature and humidity. The relative humidity was controlled at approximately 50% by bubbling a stream of air through a saturated solution of $Ca(NO_3)_2$, thoroughly filtering to remove any entrained droplets, and gently blowing the air stream over the pollen as indicated in figure 1. Room temperature was controlled within the range 25 to 27°C. The thick aluminum block on which the material was placed for irradiation served to minimize ambient temperature fluctuations by virtue of its large thermal inertia. All treatments were carried out in darkness so that possible photoreactivation processes would be minimized. The rather wide day-to-day variation in the biological response

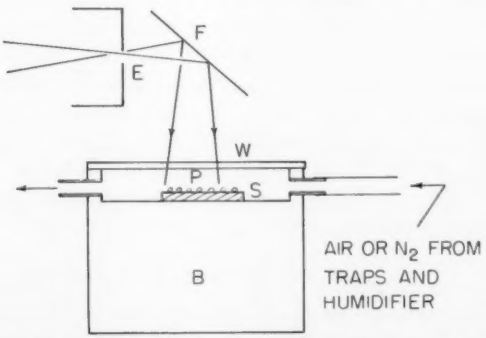

FIGURE 1.—Ultraviolet irradiation of dry pollen. E—Monochromator Exit Slit; F—Front Surface Mirror; B—Aluminum Block; S—Glass Slide; P—Pollen Monolayer; W—Fluorite Window.

of pollen to radiation previously encountered in X-ray experiments was largely avoided by these procedures and, in most cases, it has been possible to lump the results of a number of similar experiments performed at different times.

Irradiations were carried out using a monochromator with crystal quartz optics and relative aperture of f4. This instrument is relatively free from stray scattered radiation and permits slits up to 1.0 mm to be used at most wavelengths without appreciable scattered energy at the exit position. In all the present work a slit width of 0.8 mm or less was employed.

The sources used were AH4 lamps with the outer glass bulbs removed or the CH3 lamps with ultraviolet-transmitting outer envelopes. The AH4 is a better source in the 2650 Å region, although the CH3 lamp may be preferred as a radiation source in the near ultraviolet down to a wave length of 2804 Å.

The intensity of radiation delivered to the surface of the pollen grains was measured with a thermopile and galvanometer whose response was routinely calibrated against a standard lamp. The material to be irradiated was placed a few centimeters from the exit slit of the monochromator at a point where the radiation field was slightly in excess of 0.5 x 2.0 cm. Exploration of this field with the thermopile showed it to be uniform to less than 5% over the area used. The choice of the field dimensions quoted was dictated by considerations of minimum pollen sample size necessary to obtain significant numbers of scorable metaphase configurations in the processed cultures. With this area, the intensity obtained gave adequate biological effects after a short exposure at the most effective wave lengths; e.g., 20 minutes' exposure for a dose of 1×10^6 ergs/cm^2 at 2650 Å.

Studies of photoreactivation after ultraviolet irradiation were carried out by two different experimental arrangements. In the first of these, an AH4 mercury arc lamp was used in its original glass envelope mounted in a pyrex water jacket to remove undesirable infrared and ultraviolet energy. Any residual ultraviolet radiations transmitted by this system, including the 3650 Å lines of mercury, were completely removed by a Corning filter # 3389. With this arrangement, photoreactivation doses of 2×10^6 ergs/cm^2 to 5×10^8 ergs/cm^2 were given the dry pollen subsequent to ultraviolet treatment. In an attempt to discover if small amounts of 3650 Å ultraviolet radiation alone (at lower dose levels than could cause a measurable aberration rate) could result in any photorecovery, a second set of photoreactivation experiments were carried out with the monochromator assembly shown in figure 1.

Immediately after irradiation, the dry pollen was sowed on a lactose-agar medium containing colchicine and cultured 18 to 20 hours, after methods described by BISHOP (1949) and CONGER (1953). Feulgen-stained slides were made of these cultures and the chromatid aberrations scored in metaphase of the pollen tube division. All scoring was done by one person (DLC).

RESULTS AND INTERPRETATIONS

The complete experimental results are summarized in tables 1 through 5. These tables contain the dosage-aberration frequency data for (1) some typical dose-aberration curves, (2) the time-intensity, or reciprocity law studies, (3) the air-nitrogen comparison experiments, (4) the attempts to observe photoreactivation

processes, and (5) the relative effects of ultraviolet radiation of different wave lengths. Although an attempt has been made to keep the tables completely self-explanatory, some additional amplification or emphasis is needed on a few points.

Without exception, all doses were determined experimentally in a direct manner and are expressed in terms of ergs per square centimeter of ultraviolet radiation incident on the surface of the pollen grains. In all cases (with the exception of some of the photoreactivation experiments in which the filter technique was used), the dosage figures refer to monochromatic radiation of the wave length indicated. For the special photoreactivation studies, where a filter was used, the incident energy fell within the wave-length region 4000 to 6000 Å with peaks due to mercury arc lines at 4047, 4385, and 5460 Å.

In the light of the very large cytoplasmic absorption of photochemically active radiation, it is not surprising that several general physiological effects are evident in the cultured pollen, in addition to the chromosomal effects. One property of most of the ultraviolet-treated material was a much poorer germination and growth than after an amount of ionizing radiation resulting in equivalent chromosomal breakage. The results also indicate that ultraviolet-treated chromosomes are much longer and tend to clump together more than those subjected to ionizing radiation.

Chromosome fragmentation has severely limited the range over which dose-aberration frequency curves can be analyzed. This can be seen in the data of table 1 which shows the saturation in the aberration frequencies encountered in the higher doses. At still higher doses, fragmentation becomes the dominant form of chromosome damage. Under these conditions, so many cells are unscorable in the usual sense that the dose-aberration frequency curves tend to reach a limiting plateau, and scorable aberrations may even decrease with additional dose. Fragments are so numerous that any quantitative observation of them is impossible. For these reasons all experimental points used for comparison were chosen in the lower dosage ranges, where fragmentation is not a serious factor. The production of chromosome fragments by ultraviolet treatment is in sharp contrast to the effects of ionizing irradiation where, except for α-particle irradiation, very few cases of fragmentation are observed.

The data for wave-length dependence summarized in table 5 were plotted in figure 2. The curves shown here give the rough shape of the action spectrum in terms of the energy incident on the pollen grains.

TABLE 1

Variation of aberration frequency with dose

Expt. no.	UV dose [(ergs/cm²) × 10⁶]	No. of cells scored	Percentage normal	No. of aberrations per cell		
				Cd.	Iso.	Fragmented chromosomes
17, 2650 Å	0.52	400	70	0.24	0.16	0.03
	1.01	200	58	0.42	0.22	0.12
	1.52	200	49	0.50	0.29	0.18
8, 2804 Å	0.97	150	68	0.28	0.15	0
	1.99	100	47	0.57	0.44	0.19
	3.44	92	28	0.56	0.65	0.53

TABLE 2

Time-intensity data for ultraviolet-induced aberrations

Expt. no.	Intensity (ergs/cm²/sec)	UV dose, at 2650 Å [(ergs/cm²) ×10⁶]	No. of cells scored	Percentage normal cells	No. of aberrations per cell		
					Cd.	Iso.	Ex.
4	925	1.18	135	52	0.36	0.41	0.01
	93.1	1.12	25	44	0.32	0.48	0
7	958	1.15	100	41	0.61	0.49	0.01
	95.3	1.14	100	32	0.49	0.44	0
17	1092	0.51	200	71	0.22	0.17	0.01
	118	0.53	200	69	0.26	0.14	0.01

TABLE 3

Aberration frequencies for pollen irradiated in air and in nitrogen

Expt. no.	Treatment	UV dose, at 2650 Å [(ergs/cm²) × 10⁶]	Cells scored	Percentage normal cells	No. of aberrations per cell			Percentage inc. iso.
					Cd.	Iso.	Ex.	
4	Air	1.18	135	52	0.36	0.41	0.01	44
	N_2	1.15	150	53	0.37	0.39	0	66
17	Air	1.01	200	58	0.42	0.22	0.01	46
	N_2	0.98	200	56	0.37	0.24	0	65
34	Air	0.98	250	44	0.37	0.22	0.02	48
	N_2	1.00	300	35	0.45	0.21	0.01	62

TABLE 4

Results of photorecovery studies

Expt. no.	UV dose, at 2650 Å (ergs/cm²)	Photo-reactivation dose, (ergs/cm²)	Photo-reactivation wave-length Range (A)	No. of cells scored	Percentage normal	Aberration frequency per cell		
						Cd.	Iso.	Ex.
25	0.96×10^6	None		150	25	0.39	0.49	0.05
	0.98×10^6	5.37×10^8	4000	150	25	0.35	0.55	0.06
	None	5.37×10^8	4000	150	95	0.02	0.03	0
31	1.0×10^6	None		250	31	0.52	0.38	0.06
	1.0×10^6	2.98×10^6	4000	200	34	0.56	0.30	0.02
	1.0×10^6	1.49×10^7	4000	200	25	0.58	0.40	0.06
	0.99×10^6	1.47×10^6	3654	200	28	0.52	0.38	0.09
32	None	1.01×10^7	3654	225	95	0.04	0.01	0
	None	1.99×10^6	3654	50	94	0.06	0	0
	None	3.99×10^6	3654	100	96	0.05	0	0
	1.0×10^6	None		250	26	0.44	0.33	0.07
	1.0×10^6	0.82×10^7	3654	200	23	0.48	0.40	0.10

Before any real understanding of the role of ultraviolet radiation in the induction of chromosome breakage can be attained, a study of the parameters governing such damage must be made. It is particularly important that the factors influencing of modifying chromosomal breakage in pollen be determined and evaluated in much the same manner as has been done already for ionizing radiation. Taken alone, this

TABLE 5

Variation of aberration frequency with wave length

Wave length Å	UV dose (ergs/cm²)	No. of cells scored	Percentage normal	No. of aberrations per cell				RBE for isoch. aberrations normalized to unity at 2545
				Cd.	Iso.	Ex.	Fragmented chromosomes	
3654	3.01×10^7	250	88	0.10	0.03	0	0	0.024
	1.01×10^7	225	95	0.04	0.01	0	0	0.024
3130	3.25×10^7	114	92	0.02	0.06	0	0	0.043
3020	7.09×10^6	186	57	0.35	0.10	0.02	0.005	0.06
	3.57×10^6	300	69	0.27	0.09	0.03	0	0.06
2804	1×10^6	272	64	0.29	0.17	0	0.04	0.40
2650*	1×10^6	2036	40	0.43	0.33	0.04	0.61	0.79
2545	0.5×10^6	100	60	0.28	0.21	0.02	0.12	1.00
2483	0.98×10^6	350	57	0.39	0.20	0.07	0.25	0.58
2350	1.0×10^6	300	76	0.17	0.11	0.05	0.007	0.26

* Pooled data.

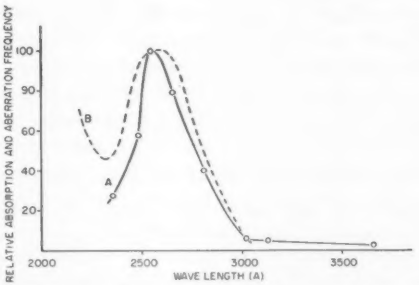

FIGURE 2.—Action spectrum for ultraviolet-induced chromosomal aberrations and nucleic acid absorption spectrum. Curve A—Action spectrum for ultraviolet-induced isochromatid aberrations. Curve B—Absorption spectrum for a nucleic acid (HOLLAENDER, GREENSTEIN and JENRETTE 1941). Ordinate units arbitrarily adjusted for equal maximum effect.

approach should yield much information for aberrations induced by ultraviolet radiation; the comparison of the results of this agent with those produced by ionizing radiation should lead to a better understanding of both phenomena. Before proceeding with the discussion of the specific individual experiments dealing with time-intensity effects, photoreactivation, air-nitrogen breakage ratios and action spectra studies, it is of interest to consider some general aspects of the data as a whole.

The experimental results show very clearly that ultraviolet radiation leads to chromosome breakage of the same types as those found for ionizing radiation. It must also be obvious that these ultraviolet-induced aberrations are the results of activation processes, since the energies available (i.e., the primary energy of the photons) are close to 5 volts for 2500 Å radiation. This is much less than the approximately 15 ev required for ionization. These figures might appear to show that activa-

tions of about 5 ev play an important part in the production of aberration by ionizing particles, for, of the 34 ev expended in producing an ion pair, almost 20 ev are dissipated in the form of activation energy. A closer examination of the ultraviolet induction of aberrations reveals that this is probably not the case, at least for radiation of wave lengths greater than 2200 Å.

This can be qualitatively shown by comparison, on an energy basis, of the effects of ultraviolet and X-rays. Experimentally, a surface dose of 2650 Å ultraviolet radiation of 10^6 ergs/cm^2 produces approximately the same number of chromatid or isochromatid aberrations as 100r of cobalt gamma rays. UBER's data (1939) for the absorption of ultraviolet radiation in maize pollen walls and cytoplasm was used for computation of a rough value for the depth dose relations in Tradescantia pollen.[2] For 2650 Å radiation, and if the generative nucleus is assumed to be located along the central axis of the pollen grain, a figure of $\sim 10^7$ to 10^8 ergs/g is obtained for a surface exposure of 10^6 ergs incident on the pollen grain.

Since the equivalent biological dose of gamma rays or hard X-rays is 100r or $\sim 10^4$ergs/g, for equal energy absorbed in the sensitive material, gamma rays are approximately 10^3 to 10^4 times as effective as ultraviolet in the production of chromosome breakage. Similar results for the depolymerization and reduction in viscosity of desoxyribonucleic acid solutions have been observed by FISHER (Personal communication 1956) in this Laboratory. Thus the relatively small amount of energy available for activation in material treated with ionizing radiation can be expected to produce a negligible biological effect compared with ionization. These arguments hold only for activations of less than 5 or 6 ev; it may be that higher energy activations have a higher relative biological efficiency. If a suitable material can be found,

[2] This estimate is arrived at by using UBER's (1939) absorption coefficients for maize pollen, assuming the absorption of ultraviolet radiation in Tradescantia pollen to be roughly equivalent to that in maize. The generative nucleus of the Tradescantia pollen grain is considered, on the average, to be at the center of the pollen grain, i.e., approximately 8 μ below the surface; the nucleus is also taken to be 4 to 5 μ in diameter. UBER's measurements on maize indicate a 20% transmission for the pollen wall and a 20% transmission for an 8-μ-thick layer of cytoplasm at 2650 Å, the wave length of maximum absorption. If these data are applied to Tradescantia, it is seen that (0.20) × (0.20) or 4% of the incident radiation reaches the generative nucleus.

The fraction of this radiation absorbed in the nucleus may be readily calculated from the usual exponential relation:

$$I/I_0 = e^{-\mu x}$$

where, I_0 = radiation incident on the pollen nucleus, I is the radiation transmitted through the pollen nucleus, and μ is the linear absorption coefficient in microns^{-1} calculated from UBER's results, 0.2 μ^{-1}.

By these values for the Tradescantia generative nucleus (taken to be 4 microns in thickness),

$$I/I_0 = e^{-0.2 \times 4} = 0.45.$$

Thus, of the 10^6 ergs/cm^2 originally incident in the pollen grain,

$$10^6 \text{ ergs/cm}^2 \times 0.04 \times 0.45 = 1.8 \times 10^4 \text{ ergs/cm}^2$$

is absorbed in the 4-μ-thick generative nucleus. In terms of ergs per gram and if the density of the nucleus is assumed to be unity, this amount of energy becomes

$$\frac{1.8 \times 10^4 \text{ ergs/cm}^2}{4 \times 10^{-4} \text{ g/cm}^2} = 4.5 \times 10^7 \text{ ergs/g}.$$

and some acceptable means for delivering ultraviolet in the vacuum range (1500 Å), a complete answer to this question may be achieved.

In contrast to the similarity in aberration types induced by ionizing and ultraviolet radiations, the relative numbers of the different aberrations produced by the two kinds of radiation are quite different. In pollen treated with 250-kvp X-rays, and at low doses (~100r) where the exchange dose-effect relations have not departed appreciably from a linear relation the ratio of chromatid to isochromatid breaks to chromatid/chromatid interchanges is approximately 1:3:1 (KIRBY-SMITH and DANIELS 1953); for this material treated with ultraviolet of wave length 2650 Å, the aberration frequencies are in the approximate ratios of 1:0.5:0.05, respectively (see tables). An additional difference is found in the numbers of the various types of isochromatid aberrations induced by ultraviolet as contrasted with X-rays. For ultraviolet treatment, the proportion of incomplete isochromatid aberrations found, i.e., isochromatid aberrations that do not show the usual fusion at both ends, is far greater than that observed for pollen irradiated with X-rays. CONGER (1955) has found that about 18% of isochromatid aberrations are incompletely fused in microspores treated with X-rays in air and nitrogen. The figures in table 3 show this to be approximately 45% in air and 65% in nitrogen for pollen exposed to ultraviolet radiation of wave length 2650 Å. These findings, the low frequency of interchanges relative to chromatid and isochromatid deletions and the high percentage of isochromatid aberrations that are incomplete, indicate that the ends of chromosomes broken by ultraviolet do not rejoin to the same extent as those produced by ionizing radiation. The extremely low aberration frequencies found for ultraviolet-induced chromatid/chromatid exchanges also explain very clearly and dramatically the infrequent occurrence of translocations so often reported in genetic studies of the action of ultraviolet radiation.

In addition, if fusion is less in breaks produced by ultraviolet than in breaks resulting from ionizing radiation, more aberrations per primary break can be expected from ultraviolet than from ionizing radiation. Since the results do show definitely that fusion is less for ultraviolet-induced breaks, the number of primary breaks per erg is even less than previously calculated (see footnote).

Considering the greatly different primary physical action of these two separate agents, ionization and excitation, major differences in the nature of the broken ends is not surprising. Excitation events, such as those induced by ultraviolet absorption, leading to chromosome breakage indicates that similar processes accompanying or resulting indirectly from ionizing radiation may be an important, or potentially important, pathway to breakage after treatment with these much more energetic agents. Conversely, the relatively small percentage of experimentally observed isochromatid aberrations due to ionizing radiation that are incomplete may be construed as evidence that the excitation process is at least not dominant in this case.

Time-intensity studies

The data accumulated in these experiments designed to determine the effects of varying ultraviolet intensity at constant dose are shown in table 2. These investigations show that the reciprocity law holds over the approximately 10:1 range in

intensities that could be studied conveniently by our existing experimental arrangements. The establishment of the validity of the reciprocity law allows a comparison of the effects of ultraviolet radiation of different wave lengths to be made independent of intensity, and greatly simplifies the practical problems encountered in constructing an action spectrum. It has been possible to make measurements of the time-intensity relations over such a range, using the mercury emission lines at 2537 and 2650 Å, both of which are close to the maximum biologically effective wave length for chromosome breakage. Although other lines in the intermediate pressure mercury spectrum are many times as intense as these, i.e., 3130 or 3650 Å, the relative biological effectiveness of energy of these wave lengths is so small that, even at the higher intensities, the time required to deliver adequate doses for a reasonable aberration frequency has prevented reciprocity law checks at other wave lengths.

Considering the relatively low exchange frequency and the small number of re-fused chromatid breaks as exemplified in the isochromatid data, one would not expect to find a great dependence on intensity for ultraviolet-induced aberrations. In other words, one-hit aberrations such as chromatid aberrations and the great majority of isochromatid aberrations are, or should be, independent of intensity, and exchanges (the intensity-dependent aberrations) are too infrequent for a practical study. If the dose is increased to a figure that should result in an appreciable interchange frequency, complete fragmentation of chromosomes or parts of chromosomes becomes so prevalent that no quantitative scoring of the results is possible.

Air-nitrogen studies

The results given (table 3) have shown that there are no significant variations in the over-all frequencies for the general types of aberrations (chromatid and isochromatid) that can be studied quantitatively, for pollen treated in air and in nitrogen. In addition these data show that within the isochromatid class the proportion of isochromatid breaks that are incomplete is much greater for pollen irradiated in nitrogen than for that treated in air. This increase, from approximately 45 to 65% incomplete, is in good agreement with the hypothesis that breaks produced in nitrogen are less likely to restitute than those produced in air. The increase is also in agreement with results for chromatid deletions in microspores treated with X-rays in air and in nitrogen (CONGER, personal communication, 1956). The additional experimental observation that equal numbers of chromatid aberrations are produced in pollen irradiated in air and in nitrogen is in complete disagreement with such a simple restitution theory. This apparent paradox can be resolved by an additional assumption that fewer breaks are initially produced in nitrogen than in air. The present experimental results thus support a tentative hypothesis in which (1) fewer breaks are produced in nitrogen than in air, and (2) these breaks induced in nitrogen are less likely to rejoin than the ones produced in material irradiated in air. This argument contains implicitly the underlying assumption that at least some of the photochemical processes involved in oxygen and in nitrogen are quite different.

Since no more aberrations are produced in pollen irradiated in air than in pollen treated in nitrogen, it appears that ozone has not been responsible for ultraviolet-induced breakage in the present experiments. These data furnish additional evidence

that ozone or ozone-producing wave lengths have been prevented from reaching the biological material.

Photorecovery processes

No photorecovery effects have been demonstrated in experiments yet performed. Table 4 shows typical results covering a wide range of dose in the wave-length region in which photorecovery has been reported in other materials. The high doses used in these experiments did not include radiation of wave length 3650 Å or less, since a very small amount of chromosomal breakage appears to have been produced at these wave lengths. Although considerable effort was made to eliminate stray scattered radiation of wave length shorter than 3650 Å, a slight possibility remains that the breakage attributed to 3650 Å may be the results of spectral impurities. Two experiments with very low doses in the region 10^6 to 10^7 ergs/cm^2 (doses that are too small to show the apparent breakage by 3650 Å radiation) also failed to show photorecovery.

Action spectrum for chromosome breakage

Having demonstrated within limits the validity of the reciprocity relations, proved that ozone is not a contributing factor in any of the present work, and showed the photorecovery processes (if present at all) are minimal effects, we have been able to proceed with less uncertainty to an investigation of the action spectrum for chromosome breakage (table 5). Owing to the possible although slight variation from day to day in pollen sensitivity, irradiations have been carried out with at least two and usually three different ultraviolet wave lengths. In this manner, by always having relative measurements for several wave lengths, it has been possible to correct for the small remaining day-to-day variations in sensitivity and construct a complete curve showing the dependence of breakage on wave length. Since only a small amount of pollen, usually enough for only one or two slides, could be treated in any one irradiation, many experiments were performed repeatedly to obtain enough metaphase figures for statistical significance.

Table 5 shows the results of a number of these independent experiments. In all but a very few experiments, irradiation of a pollen sample with a dose of 1×10^6 ergs/cm^2 or radiation of wave length 2650 Å served as a standard control point. No significant differences were noted and the 2650 Å results have been lumped as shown in the table. A curve (A) showing chromosome breakage as a function of wave length derived from these data is shown in figure 2. The absorption spectrum (curve B) for a nucleic acid (HOLLAENDER, GREENSTEIN, and JENRETTE, 1941) is included for comparison.

In all the work presented here, the ultraviolet dose considered is that dose striking the surface of the pollen grains, not the actual dose delivered to the nucleus or particular chromosomes. In the absence of definite absorption coefficient for Tradescantia pollen walls and cytoplasm, the action spectrum shown has been plotted in terms of this surface exposure. An examination of the results of UBER (1939) for ultraviolet absorption in maize pollen indicates that no major change in the general shape of the action spectrum is to be expected on application of these corrections. From

these considerations, the curve shown in figure 2 may be assumed to represent, to a good first approximation, the true action spectrum for ultraviolet-induced chromosomal breakage.

It is interesting to note that the curve (A, Fig. 2) agrees quite well with the results of STADLER and UBER (1942) for endosperm mosaics produced by ultraviolet irradiation of maize pollen. A slight discrepancy does exist in the wavelengths of maximum effectiveness between the present results for chromosome breakage and those reported by KNAPP *et al.* (1939) for genetic alteration in *Sphaerocarpus donnellii*. More precise data for the absorption and scattering of ultraviolet radiation in Tradescantia pollen and in Sphaerocarpus as well will be required before this difference can be considered significant.

SUMMARY

Dry pollen of *Tradescantia paludosa* was irradiated with monochromatic ultraviolet light of various wave lengths, and the aberrations present at the pollen tube division were studied.

Investigation of the time-intensity relations has shown that for equal doses, the intensity of incident radiation may be varied by at least a factor of 10 with no change in the frequencies for one-hit aberrations. Two-hit aberrations are too infrequent for a practical study.

Irradiation of pollen in air and in nitrogen has indicated no significant differences in the number of chromatid and isochromatid aberrations for the two treatments. This lack of an oxygen effect for ultraviolet-induced breakage is unlike the situation for ionizing radiations. The observation that the same aberration frequencies are obtained in nitrogen as in air indicates that ozone has not been responsible for the breakage in these experiments. Although the over-all frequencies were the same, the percentage of incompletely fused isochromatid aberrations increased from approximately 45 for material irradiated in air to 65 for that treated in nitrogen.

A wide range of doses of wave lengths 4000 to 6000 Å and doses of 3650 Å (smaller than that required to produce breakage) have given no photorecovery of ultraviolet-induced chromosomal damage in the dry pollen.

An action spectrum for chromosome breakage in dry Tradescantia pollen by ultraviolet light covering the range 3650 to 2350 Å has been obtained and is in good agreement with nucleic acid absorption curves.

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CONTENTS OF PRECEDING ISSUES

SEPTEMBER, 1956

Abstracts of Papers Presented at the 1956 Meetings of the Genetics Society of America.

DE SERRES, FREDERICK J., JR., Studies with purple adenine mutants in *Neurospora crassa*. I. Structural and functional complexity in the *ad-3* region.

ABRAHAMSON, S., and J. D. TELFER, The relative constancy of the X-ray-induced mutation frequency of *Drosophila melanogaster* sperm in inseminated females.

MARGOLIN, PAUL, An exception to mutual exclusion of the ciliary antigens in *Paramecium aurelia*.

MILLER, WILMER J., The hybrid-substance of the erythrocytes of the hybrids between *Columba livia* and *Streptopelia risoria*.

KIMBALL, R. F., and NENITA GAITHER, Behavior of nuclei at conjugation in *Paramecium aurelia*. II. The effects of X-rays on diploid and haploid clones, with a discussion of dominant lethals.

HILDRETH, PHILIP E., The problem of synthetic lethals in *Drosophila melanogaster*.

LEDERBERG, JOSHUA, and TEISUO IINO, Phase variation in Salmonella.

MORSE, M. L., ESTHER M. LEDERBERG, and JOSHUA LEDERBERG, Transductional heterogenotes in *Escherichia coli*.

GOWDRIDGE, BARBARA M., Heterocaryons between strains of *Neurospora crassa* with different cytoplasms.

NOVEMBER 1956

MERTENS, THOMAS R., ALLAN B. BURDICK, and F. R. GOMES, Phenotypic stability in rate of maturation of heterozygotes for induced chlorophyll mutations in tomato.

ATWOOD, K. C., R. C. VON BORSTEL, and A. R. WHITING, An influence of ploidy on the time of expression of dominant lethal mutations in Habrobracon.

EDINGTON, CHARLES W., The induction of recessive lethals in *Drosophila melanogaster* by radiations of different ion density.

MATZINGER, D. F., and O. KEMPTHORNE, The modified diallel table with partial inbreeding and interactions with environment.

SCHEINBERG, S. L., Genetic studies of cellular antigens in the chicken.

LEDERBERG, JOSHUA, Linear inheritance in transductional clones.

BRINK, R. ALEXANDER, A genetic change associated with the *R* locus in maize which is directed and potentially reversible.

KING, R. C., JEAN B. DARROW, and NANCY E. WEBER, Studies on different classes of mutations induced by radiation of *Drosophila melanogaster* females.

FRADKIN, CHENG-MEI W., and R. ALEXANDER BRINK, Crossing over in maize in the presence of the transposable factors Activator and Modulator.

BAKER, WILLIAM K., Chromosome segregation in *Drosophila virilis* males with multiple sex chromosomes.

BHAT, N. R., and N. D. DESAI, Genetic investigations of the whorled mutant in *Gossypium herbaceum*.

SAUL, GEORGE B., 2ND, and MARION KAYHART, Mutants and linkage in Mormoniella.

SPOFFORD, JANICE B., The relation between expressivity and selection against eyeless in *Drosophila melanogaster*.

JANUARY 1957

ROSTAND, JEAN, Lucien Cuénot.

FARNSWORTH, M. W., Effects of the homozygous Minute-IV deficiency on the development of *Drosophila melanogaster*.

FARNSWORTH, M. W., Effects of homozygous first, second and third chromosome minutes on the development of *Drosophila melanogaster*.

CLAYTON, FRANCES E., The effect of lozenge pseudoalleles on eye pigmentation in *Drosophila melanogaster*. I. Brown pigment and pigmentation in lozenge males.

WARD, C. L. and MARY L. ALEXANDER, Cytological analysis of X-ray-induced mutations at eight specific loci in the third chromosome of *Drosophila melanogaster*.

HINTON, CLAUDE W., The analysis of rod derivatives of an unstable ring chromosome of *Drosophila melanogaster*.

MIDDLEKAUFF, JAMES E., S. HINO, SING-PING YANG, G. and CARL C. LINDEGREN, Gene control of resistance vs. sensitivity to actidione in saccharomyces.

SAWIN, P. B., and D. D. CRARY, Morphogenetic studies of the rabbit. XVII. Disproportionate adult size induced by the *Da* gene.

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